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 NEWS  9  JUL 13  New SDI alert frequency now available in Derwent's
                  DWPI and DPCI
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                  MEDLINE
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   5 FILES SEARCHED...
   8 FILES SEARCHED...
  12 FILES SEARCHED...
  13 FILES SEARCHED...

=> S LACTIDE ANDGLYCOLIDE?
L1            0 LACTIDE ANDGLYCOLIDE?

=> S LACTIDE AND GLYCOLIDE?
L2         2550 LACTIDE AND GLYCOLIDE?

=> S L2 AND plg
L3          141 L2 AND PLG

=> s l3 and encapsula?
   7 FILES SEARCHED...
L4           98 L3 AND ENCAPSULA?
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=> s l4 and antigen
L5          50 L4 AND ANTIGEN

=> s l5 and microsphere?
L6          40 L5 AND MICROSPHERE?

=> d l6 1-40

L6   ANSWER 1 OF 40  USPATFULL
AN     2001:157849  USPATFULL
TI     Emulsion-based processes for making microparticles
IN     Gibson, John W., Springville, AL, United States
       Holl, Richard J., Indian Springs, AL, United States
       Tipton, Arthur J., Birmingham, AL, United States
PA     Southern BioSystems, Inc., Birmingham, AL, United States (U.S.
       corporation)
PI     US 6291013          B1   20010918
AI     US 1999-303842           19990503 (9)
DT     Utility
FS     GRANTED
LN.CNT 1244
INCL   INCLM: 427/213.300
       INCLS: 427/231.310; 427/213.320; 427/213.330; 427/213.360; 428/402.200;
              428/402.210; 264/004.100; 264/004.300; 264/004.330; 264/004.600
NCL    NCLM:  427/213.300
       NCLS:  427/231.310; 427/213.320; 427/213.330; 427/213.360; 428/402.200;
              428/402.210; 264/004.100; 264/004.300; 264/004.330; 264/004.600
IC     [7]
       ICM: A61K009-16
       ICS: B01J013-12
EXF    427/213.3; 427/213.31; 427/213.32; 427/213.33; 427/213.36; 428/402.2;
       428/402.21; 264/4.1; 264/4.3; 264/4.33; 264/4.6

L6   ANSWER 2 OF 40  USPATFULL
AN     2001:152520  USPATFULL
TI     Biodegradable targetable microparticle delivery system
IN     Sokoll, Kenneth K., Alton, Canada
       Chong, Pele, Richmond Hill, Canada
       Klein, Michel H., Willowdale, Canada
PA     Aventis Pasteur Limited, Toronto, Canada (non-U.S. corporation)
PI     US 6287604          B1   20010911
AI     US 2000-502674           20000211 (9)
RLI    Division of Ser. No. US 1996-770850, filed on 20 Dec 1996, now patented,
       Pat. No. US 6042820
DT     Utility
FS     GRANTED
LN.CNT 1787
INCL   INCLM: 424/501.000
       INCLS: 514/952.000; 428/402.000
NCL    NCLM:  424/501.000
       NCLS:  514/952.000; 428/402.000
IC     [7]
       ICM: A61K009-14
EXF    424/501; 514/952; 530/815; 428/402

L6   ANSWER 3 OF 40  USPATFULL
AN     2001:152505  USPATFULL
TI     Agent delivering system comprised of microparticle and biodegradable gel
       with an improved releasing profile and methods of use thereof
IN     Shih, Chung, Sandy, UT, United States
       Zentner, Gaylen M., Salt Lake City, UT, United States
PA     MacroMed, Inc., Sandy, UT, United States (U.S. corporation)
PI     US 6287588          B1   20010911
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AI US 2000-559507 20000427 (9)
PRAI US 1999-131562 19990429 (60)
DT Utility
FS GRANTED
LN.CNT 988
INCL INCLM: 424/426.000
INCLS: 424/486.000; 424/489.000; 424/501.000; 514/772.300
NCL NCLM: 424/426.000
NCLS: 424/486.000; 424/489.000; 424/501.000; 514/772.300
IC [7]
ICM: A61F002-00
ICS: A61F009-14; A61F009-50; A61F047-30
EXF 424/425; 424/423; 424/497; 424/501; 424/426; 424/486; 424/561; 424/424; 525/415
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 4 OF 40 USPATFULL
AN 2001:142135 USPATFULL
TI Zace 1: a human metalloenzyme
IN Sheppard, Paul O., Granite Falls, WA, United States
PA ZymoGenetics, Inc., Seattle, WA, United States (U.S. corporation)
PI US 6280994 B1 20010828
AI US 1999-440325 19991115 (9)
DT Utility
FS GRANTED
LN.CNT 3706
INCL INCLM: 435/226.000
INCLS: 435/069.100; 435/069.700; 435/252.300; 435/252.330; 435/320.100; 536/023.200; 536/023.400
NCL NCLM: 435/226.000
NCLS: 435/069.100; 435/069.700; 435/252.300; 435/252.330; 435/320.100; 536/023.200; 536/023.400
IC [7]
ICM: C12N015-57
ICS: C12N009-64; C12N015-74; C12N015-82; C12N015-85
EXF 435/69.1; 435/69.7; 435/226; 435/252.3; 435/252.33; 435/320.1; 536/23.2; 536/23.4
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 5 OF 40 USPATFULL
AN 2001:125581 USPATFULL
TI Method of making microencapsulated DNA for vaccination and gene therapy
IN Jones, David Hugh, Salisbury, United Kingdom
Farrar, Graham Henry, Salisbury, United Kingdom
Clegg, James Christopher Stephen, Salisbury, United Kingdom
PA Microbiological Research Authority, United Kingdom (non-U.S. corporation)
PI US 6270795 B1 20010807
AI US 1998-79400 19980515 (9)
RLI Continuation-in-part of Ser. No. US 1996-745515, filed on 12 Nov 1996
PRAI GB 1995-23019 19951109
GB 1996-1929 19960131
WO 1996-GB2770 19961111
GB 1997-9900 19970515
DT Utility
FS GRANTED
LN.CNT 1288
INCL INCLM: 424/455.000
INCLS: 424/451.000; 424/457.000; 424/484.000; 424/486.000; 424/489.000; 424/490.000; 435/320.100
NCL NCLM: 424/455.000
NCLS: 424/451.000; 424/457.000; 424/484.000; 424/486.000; 424/489.000; 424/490.000; 435/320.100
IC [7]

ICM: A61K009-66
ICS: A61K009-52; C12N015-88
EXF 514/44; 424/486; 424/489; 424/490; 424/497; 424/484; 424/451; 424/457; 424/455; 435/320.1; 435/455; 435/422; 435/425; 435/426
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 6 OF 40 USPATFULL
AN 2001:93325 USPATFULL
TI Sequence and method for genetic engineering of proteins with cell membrane translocating activity
IN Lin, Yao-Zhong, Nashville, TN, United States
Donahue, John P., Nashville, TN, United States
Rojas, Mauricio, Nashville, TN, United States
Tan, Zhong-Jia, Nashville, TN, United States
PA Vanderbilt University, Nashville, TN, United States (U.S. corporation)
PI US 6248558 B1 20010619
AI US 1998-186170 19981104 (9)
PRAI US 1998-80083 19980331 (60)
DT Utility
FS GRANTED
LN.CNT 1376
INCL INCLM: 435/069.100
INCLS: 536/023.100; 435/320.100; 514/012.000
NCL NCLM: 435/069.100
NCLS: 435/320.100; 514/012.000; 536/023.100
IC [7]
ICM: C12P021-06
ICS: C07H021-02; C12N015-00; A61K038-00
EXF 536/23.1; 435/320.1; 435/69.1; 514/12
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 7 OF 40 USPATFULL
AN 2001:88962 USPATFULL
TI Hepatitis B core **antigen** nucleic acid vaccine
IN Lu, Shan, Northborough, MA, United States
Huang, Zuhu, Nanjing, China
Herrmann, John E., Northborough, MA, United States
PA University of Massachusetts, Massachusetts Corporation (U.S. corporation)
PI US 2001001098 A1 20010510
AI US 2001-756500 A1 20010108 (9)
RLI Continuation of Ser. No. US 1999-400497, filed on 21 Sep 1999, ABANDONED
PRAI US 1998-101311 19980921 (60)
DT Utility
FS APPLICATION
LN.CNT 596
INCL INCLM: 514/044.000
INCLS: 424/093.210
NCL NCLM: 514/044.000
NCLS: 424/093.210
IC [7]
ICM: A61K031-70
ICS: A01N043-04; A61K048-00; A01N063-00
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 8 OF 40 USPATFULL
AN 2001:67234 USPATFULL
TI Biodegradable targetable microparticle delivery system
IN Sokoll, Kenneth K., Alton, Canada
Chong, Pele, Richmond Hill, Canada
Klein, Michel H., Willowdale, Canada
PA Connaught Laboratories Limited, North York, Canada (non-U.S. corporation)
PI US 6228423 B1 20010508

AI US 2000-501373 20000211 (9)
RLI Division of Ser. No. US 1996-770850, filed on 20 Dec 1996, now patented, Pat. No. US 6042820
DT Utility
FS Granted
LN.CNT 1765
INCL INCLM: 427/213.300
INCLS: 427/213.340
NCL NCLM: 427/213.300
NCLS: 427/213.340
IC [7]
ICM: B01J013-02
ICS: B05D007-00
EXF 427/213.3; 427/213.34
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 9 OF 40 USPATFULL
AN 2001:21767 USPATFULL
TI Cross-protective rotavirus vaccine
IN Herrmann, John E., Northborough, MA, United States
Lu, Shan, Northborough, MA, United States
PA University of Massachusetts, Boston, MA, United States (U.S. corporation)
PI US 6187319 B1 20010213
AI US 1998-88216 19980601 (9)
DT Utility
FS Granted
LN.CNT 724
INCL INCLM: 424/215.100
INCLS: 514/044.000
NCL NCLM: 424/215.100
NCLS: 514/044.000
IC [7]
ICM: A61K039-15
EXF 424/204.1; 424/215.1; 514/44
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 10 OF 40 USPATFULL
AN 2000:87732 USPATFULL
TI Use of microparticles combined with submicron oil-in-water emulsions
IN O'Hagan, Derek, Berkeley, CA, United States
Van Nest, Gary, El Sobrante, CA, United States
Ott, Gary S., Oakland, CA, United States
Singh, Manmohan, Hercules, CA, United States
PA Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
PI US 6086901 20000711
AI US 1998-15736 19980129 (9)
PRAI US 1997-69724 19971216 (60)
DT Utility
FS Granted
LN.CNT 1127
INCL INCLM: 424/283.100
INCLS: 424/070.110; 424/070.190; 424/204.100; 424/228.100; 424/278.100; 424/280.000
NCL NCLM: 424/283.100
NCLS: 424/070.110; 424/070.190; 424/204.100; 424/228.100; 424/278.100; 424/497.000
IC [7]
ICM: A61K039-29
ICS: A61K007-08; A61K045-00; A61K047-44
EXF 424/278.1; 424/280.1; 424/283.1; 424/70.11; 424/70.19; 424/204.1; 424/228.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 11 OF 40 USPATFULL
AN 2000:50377 USPATFULL
TI Hapten-carrier conjugates for use in drug-abuse therapy and methods for preparation of same
IN Swain, Philip A., Boston, MA, United States
Schad, Victoria C., Cambridge, MA, United States
Greenstein, Julia L., West Newton, MA, United States
Exley, Mark A., Chestnut Hill, MA, United States
Fox, Barbara S., Wayland, MA, United States
Powers, Stephen P., Waltham, MA, United States
Gefter, Malcolm L., Lincoln, MA, United States
Briner, Thomas J., Arlington, MA, United States
PA Immulogic Pharmaceutical Corporation, Waltham, MA, United States (U.S. corporation)
PI US 6054127 20000425
AI US 1997-884497 19970627 (8)
RLI Division of Ser. No. US 1995-563673, filed on 28 Nov 1995, now patented, Pat. No. US 5760184 which is a continuation-in-part of Ser. No. US 1995-414971, filed on 31 Mar 1995, now abandoned
DT Utility
FS Granted
LN.CNT 2598
INCL INCLM: 424/194.100
INCLS: 424/236.100; 424/204.100; 424/261.100; 530/403.000; 530/405.000; 546/124.000; 546/129.000; 546/130.000; 546/132.000
NCL NCLM: 424/194.100
NCLS: 424/204.100; 424/236.100; 424/261.100; 530/403.000; 530/405.000; 546/124.000; 546/129.000; 546/130.000; 546/132.000
IC [7]
ICM: A61K039-385
ICS: C07D451-02
EXF 424/193.1; 424/130.1; 424/175.1; 424/194.1; 424/196.11; 424/236.1; 424/197.11; 424/204.1; 424/261.1; 530/403; 530/405; 530/345; 530/387.1; 530/389.8; 546/129; 546/132; 546/121; 546/130
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 12 OF 40 USPATFULL
AN 2000:37378 USPATFULL
TI Biodegradable copolymer containing .alpha.-hydroxy acid and .alpha.-amino acid units
IN Sokoll, Kenneth K., Alton, Canada
Chong, Pele, Richmond Hill, Canada
Klein, Michel H., Willowdale, Canada
PA Connaught Laboratories Limited, North York, Canada (non-U.S. corporation)
PI US 6042820 20000328
AI US 1996-770850 19961220 (8)
DT Utility
FS Granted
LN.CNT 1774
INCL INCLM: 424/078.300
INCLS: 424/078.370; 514/772.700; 525/415.000; 528/354.000; 528/357.000; 528/359.000; 528/380.000; 530/815.000; 530/816.000
NCL NCLM: 424/078.300
NCLS: 424/078.370; 514/772.700; 525/415.000; 528/354.000; 528/357.000; 528/359.000; 528/380.000; 530/815.000; 530/816.000
IC [7]
ICM: A61K031-765
ICS: A61K047-34; C08G063-08
EXF 424/78.3; 424/78.37; 514/772.7; 525/415; 525/410; 525/411; 525/413; 528/353; 528/354; 528/357; 528/359; 528/380; 530/815; 530/816
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 13 OF 40 USPATFULL

AN 2000:18071 USPATFULL
TI Composition for delivering bioactive agents for immune response and its preparation
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The Uab Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 6024983 20000215
AI US 1993-116802 19930907 (8)
RLI Continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2328
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/499.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/889.000; 514/958.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/499.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/889.000; 514/958.000; 514/963.000
IC [7]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/237.1; 424/256.1; 424/434; 424/439; 424/497; 424/499; 424/810; 424/501; 514/885; 514/889; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 14 OF 40 USPATFULL
AN 2000:4432 USPATFULL
TI Methods for enhancement of protective immune responses
IN Reed, Steven G., Bellevue, WA, United States
PA Corixa Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6013268 20000111
AI US 1997-989370 19971212 (8)
RLI Continuation-in-part of Ser. No. US 1996-634642, filed on 18 Apr 1996, now patented, Pat. No. US 5879687, issued on 9 Mar 1999 which is a continuation-in-part of Ser. No. US 1996-607509, filed on 23 Feb 1996, now patented, Pat. No. US 5876735, issued on 2 Mar 1999 which is a continuation-in-part of Ser. No. US 1995-488386, filed on 6 Jun 1995, now abandoned which is a continuation-in-part of Ser. No. US 1995-454036, filed on 30 May 1995, now patented, Pat. No. US 5876966, issued on 2 Mar 1999 which is a continuation-in-part of Ser. No. US 1994-232534, filed on 22 Apr 1994, now abandoned
DT Utility
FS Granted
LN.CNT 2882
INCL INCLM: 424/269.100
INCLS: 424/184.100; 424/450.000; 424/265.100; 530/350.000; 536/023.100; 514/012.000; 514/044.000
NCL NCLM: 424/269.100
NCLS: 424/184.100; 424/265.100; 424/450.000; 514/012.000; 514/044.000; 530/350.000; 536/023.100
IC [6]
ICM: A61K048-00

ICS: A61K039-00; A61K031-70; C07K014-00
EXF 530/350; 424/269.1; 424/450; 424/184.1; 424/265.1; 536/23.1; 536/23.7; 514/12; 514/44
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 15 OF 40 USPATFULL
AN 1999:163251 USPATFULL
TI Polymeric lamellar substrate particles for drug delivery
IN Coombes, Allan Gerald Arthur, Nottingham, United Kingdom
Davis, Stanley Stewart, Nottingham, United Kingdom
Major, Diane Lisa, London, United Kingdom
Wood, John Michael, Hertsfordshire, United Kingdom
PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)
PI US 6001395 19991214
WO 9702810 19970130
AI US 1998-983156 19980330 (8)
WO 1996-GB1695 19960715
19980330 PCT 371 date
19980330 PCT 102(e) date
PRAI GB 1995-14285 19950713
DT Utility
FS Granted
LN.CNT 793
INCL INCLM: 424/501.000
INCLS: 424/426.000; 424/490.000
NCL NCLM: 424/501.000
NCLS: 424/426.000; 424/490.000
IC [6]
ICM: A61K009-16
ICS: A61K047-34
EXF 424/486; 424/426; 424/458; 424/428; 424/459; 424/490; 424/501; 514/952; 428/402; 428/402.24; 427/2.14
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 16 OF 40 USPATFULL
AN 1999:99400 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5942252 19990824
AI US 1995-469463 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2060
INCL INCLM: 424/501.000
INCLS: 424/426.000; 424/430.000; 424/434.000; 424/435.000; 424/436.000; 424/451.000; 424/464.000
NCL NCLM: 424/501.000
NCLS: 424/426.000; 424/430.000; 424/434.000; 424/435.000; 424/436.000; 424/451.000; 424/464.000

IC [6]
ICM: A61K009-50
ICS: A61K009-48; A61F002-02; A61F009-02
EXF 424/489; 424/451; 424/464; 424/490; 424/426; 424/430; 424/434; 424/435; 424/436; 424/501; 514/772.3; 514/912
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 17 OF 40 USPATFULL
AN 1999:56240 USPATFULL
TI Spray dried vaccine preparation comprising aluminium adsorbed immunogens
IN Cox, John Cooper, Bullengarook, Australia
Sparks, Robert Edward, Kirkwood, MO, United States
Jacobs, Irwin Clay, Eureka, MO, United States
Mason, Norbert Simon, St. Louis, MO, United States
PA CSL Limited, Parkville, Australia (non-U.S. corporation)
PI US 5902565 19990511
WO 9415636 19940721
AI US 1995-481403 19950710 (8)
WO 1993-AU677 19931224
19950710 PCT 371 date
19950710 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 2485
DT Utility
FS Granted
LN.CNT 871
INCL INCLM: 424/001.290
INCLS: 424/001.330; 424/489.000; 424/499.000; 424/457.000; 424/460.000; 424/461.000
NCL NCLM: 424/001.290
NCLS: 424/001.330; 424/457.000; 424/460.000; 424/461.000; 424/489.000; 424/499.000
IC [6]
ICM: A61K051-00
ICS: A61K009-16; A61K009-50; A61K009-60
EXF 424/489; 424/457; 424/460; 424/461; 424/1.29; 424/1.33; 424/499
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 18 OF 40 USPATFULL
AN 1999:30376 USPATFULL
TI Methods for enhancement of protective immune responses
IN Reed, Steven G., Bellevue, WA, United States
PA Corixa Corporation, Seattle, WA, United States (U.S. corporation)
PI US 5879687 19990309
AI US 1996-634642 19960418 (8)
RLI Continuation-in-part of Ser. No. US 1996-607509, filed on 23 Feb 1996 which is a continuation-in-part of Ser. No. US 1995-488386, filed on 6 Jun 1995, now abandoned which is a continuation-in-part of Ser. No. US 1994-232534, filed on 22 Apr 1994, now abandoned
DT Utility
FS Granted
LN.CNT 2192
INCL INCLM: 424/269.100
INCLS: 424/184.100; 514/012.000
NCL NCLM: 424/269.100
NCLS: 424/184.100; 514/012.000
IC [6]
ICM: A61K039-008
ICS: A61K039-39; C07K014-44; C12N015-30
EXF 424/184.1; 424/269.1; 514/12
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 19 OF 40 USPATFULL
AN 1999:27202 USPATFULL
TI Methods for enhancement of protective immune responses

IN Reed, Steven G., Bellevue, WA, United States
PA Corixa Corporation, Seattle, WA, United States (U.S. corporation)
PI US 5876735 19990302
AI US 1996-607509 19960223 (8)
RLI Continuation-in-part of Ser. No. US 1995-488386, filed on 6 Jun 1995, now abandoned which is a continuation-in-part of Ser. No. US 1994-232534, filed on 22 Apr 1994, now abandoned
DT Utility
FS Granted
LN.CNT 2193
INCL INCLM: 424/269.100
INCLS: 424/184.100; 424/450.000; 514/012.000
NCL NCLM: 424/269.100
NCLS: 424/184.100; 424/450.000; 514/012.000
IC [6]
ICM: A61K039-00
ICS: A61K039-002; A61K039-008; A61K009-127
EXF 424/269.1; 424/184.1; 424/450; 514/12
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 20 OF 40 USPATFULL
AN 1999:27194 USPATFULL
TI Hapten-carrier conjugates for use in drug-abuse therapy and methods for preparation of same
IN Swain, Philip A., Boston, MA, United States
Schad, Victoria C., Cambridge, MA, United States
Greenstein, Julia L., West Newton, MA, United States
Exley, Mark A., Chestnut Hill, MA, United States
Fox, Barbara S., Wayland, MA, United States
Powers, Stephen P., Waltham, MA, United States
Gefter, Malcolm L., Lincoln, MA, United States
PA ImmuLogic Pharmaceutical Corporation, Waltham, MA, United States (U.S. corporation)
PI US 5876727 19990302
AI US 1996-720487 19960930 (8)
RLI Continuation-in-part of Ser. No. US 1995-563673, filed on 28 Nov 1995, now patented, Pat. No. US 5760184 which is a continuation-in-part of Ser. No. US 1995-414971, filed on 31 Mar 1995, now abandoned
DT Utility
FS Granted
LN.CNT 3369
INCL INCLM: 424/193.100
INCLS: 424/130.100; 424/175.100; 424/194.100; 546/129.000; 546/130.000; 546/131.000; 546/132.000; 546/279.400; 514/343.000; 530/405.000; 530/409.000
NCL NCLM: 424/193.100
NCLS: 424/130.100; 424/175.100; 424/194.100; 514/343.000; 530/405.000; 530/409.000; 546/129.000; 546/130.000; 546/131.000; 546/132.000; 546/279.400
IC [6]
ICM: A61K039-385
ICS: A61K039-395; C07D451-02
EXF 530/405; 530/409; 424/130.1; 424/175.1; 424/193.1; 424/194.1; 546/129; 546/130; 546/131; 546/132; 546/279.4; 514/343
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 21 OF 40 USPATFULL
AN 1999:18774 USPATFULL
TI Polymer microparticles for drug delivery
IN Yeh, Ming-Kung, Taipei, Taiwan, Province of China
Coombes, Alan Gerald, Nottingham, United Kingdom
Jenkins, Paul George, Macclesfield, United Kingdom
Davis, Stanley Stewart, Nottingham, United Kingdom
PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)

PI US 5869103 19990209
WO 9535097 19951228
AI US 1997-750738 19970404 (8)
WO 1995-GB1426 19950619
19970404 PCT 371 date
19970404 PCT 102(e) date
PRAI GB 1994-12273 19940618
DT Utility
FS Granted
LN.CNT 1058
INCL INCLM: 424/501.000
INCLS: 424/502.000; 264/004.100; 264/004.600
NCL NCLM: 424/501.000
NCLS: 264/004.100; 264/004.600; 424/502.000
IC [6]
ICM: A61K009-50
ICS: B01J013-02
EXF 424/501; 424/502; 264/4.1; 264/4.6
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 22 OF 40 USPATFULL
AN 1998:162037 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissue and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5853763 19981229
AI US 1995-467314 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2263
INCL INCLM: 424/489.000
INCLS: 424/184.100; 424/204.100; 424/206.100; 424/234.100; 424/237.100; 424/434.000; 424/435.000; 424/436.000; 424/499.000; 424/501.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/489.000
NCLS: 424/184.100; 424/204.100; 424/206.100; 424/234.100; 424/237.100; 424/434.000; 424/435.000; 424/436.000; 424/499.000; 424/501.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/435; 424/501; 424/489; 514/888; 514/963; 514/885; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 23 OF 40 USPATFULL
AN 1998:147035 USPATFULL
TI Hapten-carrier conjugates for use in drug-abuse therapy and methods for preparation

IN Swain, Philip A., Brighton, MA, United States
Schad, Victoria Carol, Cambridge, MA, United States
Greenstein, Julia Lea, West Newton, MA, United States
Exley, Mark Adrian, Brookline, MA, United States
Fox, Barbara Saxton, Wayland, MA, United States
Powers, Stephen P., Waltham, MA, United States
Gefter, Malcolm L., Lincoln, MA, United States
PA ImmuLogic Pharmacuetical Corp., Waltham, MA, United States (U.S. corporation)
PI US 5840307 19981124
AI US 1995-457206 19950601 (8)
RLI Division of Ser. No. US 1995-414971, filed on 31 Mar 1995
DT Utility
FS Granted
LN.CNT 2082
INCL INCLM: 424/193.100
INCLS: 424/140.100; 424/175.100; 546/130.000
NCL NCLM: 424/193.100
NCLS: 424/140.100; 424/175.100; 546/130.000
IC [6]
ICM: A61K039-385
ICS: A61K039-00; A61K039-395; C07P451-02
EXF 424/193.1; 424/140.1; 424/175.1; 546/130
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 24 OF 40 USPATFULL
AN 1998:124217 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5820883 19981013
AI US 1995-468064 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2355
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/810; 424/501; 514/888; 514/497; 514/885; 514/958; 514/963; 514/810; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 25 OF 40 USPATFULL

AN 1998:118870 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5814344 19980929
AI US 4692187 19950606 (8)
RLI Continuation of Ser. No. 116484, filed on 7 Sep 1993 which is a continuation of Ser. No. 629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. 325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. 169973, filed on 18 Mar 1988, now patented, Pat. No. 5075109 which is a continuation-in-part of Ser. No. 923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2121
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/501; 514/499; 514/888; 514/963; 514/885; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 26 OF 40 USPATFULL
AN 1998:115447 USPATFULL
TI Method for oral or rectal delivery of microencapsulated vaccines and compositions therefor
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5811128 19980922
AI US 1164848 19930907 (8)
RLI Continuation of Ser. No. 629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. 325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. 169973, filed on 18 Mar 1988, now patented, Pat. No. 5075109 which is a continuation-in-part of Ser. No. 923159, filed on 24 Oct 1996, now abandoned
DT Utility
FS Granted
LN.CNT 2353
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 428/402.210; 428/202.240; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000

NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/501; 514/888; 514/963; 514/885; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 27 OF 40 USPATFULL
AN 1998:75160 USPATFULL
TI Hapten-carrier conjugates for use in drug-abuse therapy and methods for preparation of same
IN Swain, Philip A., Brighton, MA, United States
Schad, Victoria Carol, Cambridge, MA, United States
Greenstein, Julia Lea, West Newton, MA, United States
Exley, Mark Adrian, Chestnut Hill, MA, United States
Fox, Barbara Saxton, Wayland, MA, United States
Powers, Stephen P., Waltham, MA, United States
Gefter, Malcolm L., Lincoln, MA, United States
PA ImmuLogic, Inc., Waltham, MA, United States (U.S. corporation)
PI US 5773003 19980630
AI US 1995-456444 19950601 (8)
RLI Division of Ser. No. US 1995-414971, filed on 31 Mar 1995, now abandoned
DT Utility
FS Granted
LN.CNT 2144
INCL INCLM: 424/193.100
INCLS: 424/175.100; 424/194.100; 424/196.110; 424/204.100
NCL NCLM: 424/193.100
NCLS: 424/175.100; 424/194.100; 424/196.110; 424/204.100
IC [6]
ICM: A61K039-385
ICS: A61K039-12
EXF 424/193.1; 424/130.1; 424/175.1; 424/194.1; 424/196.11; 424/204.1; 530/404; 530/405; 530/408; 530/409; 546/129-132
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 28 OF 40 USPATFULL
AN 1998:64760 USPATFULL
TI Vaccines against intracellular pathogens using antigens **encapsulated** within biodegradble-biocompatible **microspheres**
IN Burnett, Paul R., Silver Spring, MD, United States
Van Hamont, John E., Ft. Meade, MD, United States
Reid, Robert H., Kensington, MD, United States
Setterstrom, Jean A., Alpharetta, GA, United States
Van Cott, Thomas C., Brookeville, MD, United States
Birx, Debrah L., Potomac, MD, United States
PA The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
PI US 5762965 19980609
AI US 1996-598874 19960209 (8)
RLI Continuation-in-part of Ser. No. US 1994-242960, filed on 16 May 1994 And Ser. No. US 1995-446149, filed on 22 May 1995 which is a continuation of Ser. No. US 1984-590308, filed on 16 Mar 1984, now abandoned , said Ser. No. US -242960 which is a continuation-in-part of Ser. No. US 1992-867301, filed on 10 Apr 1992, now patented, Pat. No. US 5417986 which is a continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990, now abandoned

DT Utility
FS Granted
LN.CNT 315
INCL INCLM: 424/499.000
INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/422.000
NCL NCLM: 424/499.000
NCLS: 424/422.000; 424/426.000; 424/455.000; 424/486.000; 424/488.000
IC [6]
ICM: A61K009-00
ICS: A61K009-66; A61K009-14; A61F013-00
EXF 424/499; 424/426; 424/455; 424/486; 424/488; 424/422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 29 OF 40 USPATFULL
AN 1998:61797 USPATFULL
TI Hapten-carrier conjugates for use in drug-abuse therapy and methods for preparation of same
IN Swain, Philip A., Boston, MA, United States
Schad, Victoria C., Cambridge, MA, United States
Greenstein, Julia L., West Newton, MA, United States
Exley, Mark A., Chestnut Hill, MA, United States
Fox, Barbara S., Wayland, MA, United States
Powers, Stephen P., Waltham, MA, United States
Gefter, Malcolm L., Lincoln, MA, United States
Briner, Thomas J., Arlington, MA, United States
PA ImmuLogic, Inc., Waltham, MA, United States (U.S. corporation)
PI US 5760184 19980602
AI US 1995-563673 19951128 (8)
RLI Continuation-in-part of Ser. No. US 1995-414971, filed on 30 Mar 1995, now abandoned
DT Utility
FS Granted
LN.CNT 2609
INCL INCLM: 530/387.100
INCLS: 530/389.800; 424/193.100; 424/175.100; 424/236.100; 424/261.100; 424/196.110; 424/197.110
NCL NCLM: 530/387.100
NCLS: 424/175.100; 424/193.100; 424/196.110; 424/197.110; 424/236.100; 424/261.100; 530/389.800
IC [6]
ICM: C07K016-00
ICS: A61K039-385; A61K039-395
EXF 424/193.1; 424/175.1; 424/196.11; 424/197.11; 424/236.1; 424/261.1; 424/204.1; 530/387.1; 530/389.8; 546/112
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 30 OF 40 USPATFULL
AN 1998:39566 USPATFULL
TI Biodegradable in-situ forming implants and methods of producing the same
IN Dunn, Richard L., Fort Collins, CO, United States
English, James P., Birmingham, AL, United States
Cowsar, Donald R., Birmingham, AL, United States
Vanderbilt, David D., Birmingham, AL, United States
PA Atrix Laboratories, Inc., Fort Collins, CO, United States (U.S. corporation)
PI US 5739176 19980414
AI US 1994-210891 19940318 (8)
RLI Continuation of Ser. No. US 1991-788032, filed on 23 Dec 1991, now patented, Pat. No. US 5340849, issued on 23 Aug 1994 which is a division of Ser. No. US 1990-513782, filed on 24 Apr 1990, now patented, Pat. No. US 5278201 which is a division of Ser. No. US 1988-252645, filed on 3 Oct 1988, now patented, Pat. No. US 4938763, issued on 3 Jul 1990
DT Utility
FS Granted

LN.CNT 1210
INCL INCLM: 523/113.000
INCLS: 523/115.000; 604/051.000; 604/054.000; 604/056.000; 604/290.000; 525/408.000; 525/412.000; 525/413.000; 525/937.000; 424/422.000; 424/078.380; 524/096.000; 524/601.000
NCL NCLM: 523/113.000
NCLS: 424/078.380; 424/422.000; 523/115.000; 524/096.000; 524/601.000; 525/408.000; 525/412.000; 525/413.000; 525/937.000; 604/290.000; 604/506.000
IC [6]
ICM: A61F002-02
ICS: A61F002-00
EXF 523/113; 523/115; 524/96; 524/601; 525/937; 525/408; 525/412; 525/413; 424/422; 424/78.38; 604/51; 604/54; 604/26; 604/290
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 31 OF 40 USPATFULL
AN 97:112180 USPATFULL
TI Microparticle carriers of maximal uptake capacity by both M cells and non-M cells
IN Reid, Robert H., Kensington, MD, United States
van Hamont, John E., Fort Meade, MD, United States
Brown, William R., Denver, CO, United States
Boedeker, Egar C., Chevy Chase, MD, United States
Thies, Curt, Ballwin, MO, United States
PA The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
PI US 5693343 19971202
AI US 1994-242960 19940516 (8)
RLI Continuation-in-part of Ser. No. US 1992-867301, filed on 10 Apr 1992, now patented, Pat. No. US 5417986 which is a continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990, now abandoned which is a continuation-in-part of Ser. No. US 1990-493597, filed on 15 Mar 1990, now abandoned which is a continuation-in-part of Ser. No. US 1984-590308, filed on 16 Mar 1984
DT Utility
FS Granted
LN.CNT 624
INCL INCLM: 424/491.000
INCLS: 424/493.000; 424/486.000; 424/497.000; 424/499.000; 424/501.000; 514/788.100; 514/965.000
NCL NCLM: 424/491.000
NCLS: 424/486.000; 424/493.000; 424/497.000; 424/499.000; 424/501.000; 514/788.100; 514/965.000
IC [6]
ICM: A61K009-16
ICS: A61K009-50; A61K047-30
EXF 424/491; 424/493; 424/486; 424/497; 424/499; 424/501; 424/DIG.7; 514/965
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 32 OF 40 USPATFULL
AN 97:14439 USPATFULL
TI Preparation of microparticles and method of immunization
IN O'Hagan, Derek T., 16 Middlesex Rd., Bootle, Merseyside L20 9BW, United Kingdom
McGee, John P., Tanjong Kilmarnack Rd., Kilmaurs, Strathelyde KA3 2RB, Scotland
Davis, Stanley S., 19 Cavendish Crescent North, Nottingham NG7 1BA, United Kingdom
PI US 5603960 19970218
WO 9427718 19941208

AI US 1995-374751 19950602 (8)
WO 1994-US5834 19940524
19950602 PCT 371 date
19950602 PCT 102(e) date
PRAI GB 1993-10781 19930525
DT Utility
FS Granted
LN.CNT 789
INCL INCLM: 424/501.000
INCLS: 424/451.000; 424/489.000; 264/004.100; 428/402.210; 428/402.240; 514/885.000; 514/963.000; 530/806.000
NCL NCLM: 424/501.000
NCLS: 264/004.100; 424/451.000; 424/489.000; 428/402.210; 428/402.240; 514/885.000; 514/963.000; 530/806.000
IC [6]
ICM: A61K009-50
ICS: A61K009-48; A61K009-14; B01J013-02
EXF 424/451; 424/489; 424/501; 264/4.1; 428/402.21; 428/402.24; 514/885; 514/963; 530/806
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 33 OF 40 USPATFULL
AN 95:45359 USPATFULL
TI Vaccines against diseases caused by enteropathogenic organisms using antigens **encapsulated** within biodegradable-biocompatible **microspheres**
IN Reid, Robert H., Kensington, MD, United States
Boedeker, Edgar C., Chevy Chase, MD, United States
van Hamont, John E., Shape, Belgium
Setterstrom, Jean A., Takoma Park, MD, United States
PA The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
PI US 5417986 19950523
AI US 1992-867301 19920410 (7)
RLI Continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990, now abandoned which is a continuation-in-part of Ser. No. US 1990-493597, filed on 15 Mar 1990, now abandoned which is a continuation-in-part of Ser. No. US 1984-590308, filed on 16 Mar 1984
DT Utility
FS Granted
LN.CNT 2736
INCL INCLM: 424/499.000
INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/489.000; 424/444.000; 424/433.000; 424/470.000; 424/491.000; 424/422.000
NCL NCLM: 424/499.000
NCLS: 424/422.000; 424/426.000; 424/433.000; 424/444.000; 424/455.000; 424/470.000; 424/486.000; 424/488.000; 424/489.000; 424/491.000
IC [6]
ICM: A61K009-50
ICS: A61K009-66; A61K009-26
EXF 424/499; 424/422; 424/85; 424/417; 424/450; 424/458; 424/469; 424/88; 424/89; 424/92; 424/863; 424/965
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 34 OF 40 USPATFULL
AN 94:55340 USPATFULL
TI Intragingival delivery systems for treatment of periodontal disease
IN Dunn, Richard L., Fort Collins, CO, United States
Tipton, Arthur J., Fort Collins, CO, United States
Harkrader, Ronald J., Louisville, CO, United States
Rogers, Jack A., Fort Collins, CO, United States

PA Vipont Pharmaceutical, Inc., New York, NY, United States (U.S. corporation)
PI US 5324520 19940628
AI US 1993-46396 19930413 (8)
RLI Continuation of Ser. No. US 1991-742719, filed on 5 Aug 1991, now abandoned which is a continuation of Ser. No. US 1988-286456, filed on 19 Dec 1988, now abandoned
DT Utility
FS Granted
LN.CNT 462
INCL INCLM: 424/435.000
INCLS: 424/426.000; 424/434.000; 424/450.000; 424/451.000; 424/486.000; 424/487.000; 424/489.000; 424/490.000; 436/829.000; 514/953.000; 514/963.000; 264/004.100; 264/004.330; 264/004.600; 264/004.700
NCL NCLM: 424/435.000
NCLS: 264/004.100; 264/004.330; 264/004.600; 264/004.700; 424/426.000; 424/434.000; 424/450.000; 424/451.000; 424/486.000; 424/487.000; 424/489.000; 424/490.000; 436/829.000; 514/953.000; 514/963.000
IC [5]
ICM: A61K009-14
ICS: A61K009-16; A61K009-48; A61K037-22
EXF 424/422; 424/426; 424/435; 424/434; 424/486; 424/487; 424/451; 424/489; 424/490; 264/4.1; 264/4.33; 264/4.6; 264/4.7; 514/772.3; 514/963; 514/450; 514/953; 436/829
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 35 OF 40 USPATFULL
AN 92:27517 USPATFULL
TI Controlled-release formulations of interleukin-2
IN Singh, Maninder, Mountain Brook, Rodeo, CA, United States
Nunberg, Jack H., Mountain Brook, Oakland, CA, United States
Tice, Thomas R., Mountain Brook, Birmingham, AL, United States
Hudson, Michael E., Mountain Brook, Gardendale, AL, United States
Gilley, Richard M., Mountain Brook, AL, CA, United States
Taforo, Terrance A., San Leandro, CA, United States
PA Cetus Corporation, Emeryville, CA, United States (U.S. corporation)
PI US 5102872 19920407
AI US 1988-231757 19880812 (7)
RLI Continuation-in-part of Ser. No. US 1986-856680, filed on 25 Apr 1986, now patented, Pat. No. US 4818769 which is a continuation-in-part of Ser. No. US 1985-778371, filed on 20 Sep 1985, now abandoned
DT Utility
FS Granted
LN.CNT 883
INCL INCLM: 514/021.000
INCLS: 514/002.000; 514/963.000; 514/921.000; 514/872.000; 514/012.000; 930/141.000; 424/499.000
NCL NCLM: 514/021.000
NCLS: 424/499.000; 514/002.000; 514/012.000; 514/872.000; 514/921.000; 514/963.000; 930/141.000
IC [5]
ICM: A61K037-02
EXF 514/12; 514/2; 514/921; 514/872; 514/963; 424/499; 930/141
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 36 OF 40 ADISALERTS COPYRIGHT 2001 (ADIS)
AN 1994:36344 ADISALERTS
DN 800262648
TI New strategies for using mucosal vaccination to achieve more effective immunization
ADIS TITLE: Vaccines: pharmacodynamics.; Strategies of mucosal vaccination; Review (149 references)
AU Walker R I
CS National Vaccine Program Office, Rockville, Maryland, USA; Medical

Biotechnology Center, Baltimore, Maryland, USA
SO Vaccine (Apr 1, 1994), Vol. 12, pp. 387-400
DT General Review
RE Vaccines (Summary): Alert no. 5, 1994
FS Summary
LA English
WC 1242

L6 ANSWER 37 OF 40 ADISINSIGHT COPYRIGHT 2001 (ADIS)
ACCESSION NUMBER: 1998:5495 ADISINSIGHT
SOURCE: Adis R&D Insight
DOCUMENT NO: 006108
CHANGE DATE: Jul 18, 2000
GENERIC NAME: Venezuelan equine encephalitis virus vaccine
SYNONYM: V3526
MOLECULAR FORMULA:Unspecified
STRUCTURE:
STRUCTURE DIAGRAM IS NOT AVAILABLE

EPHMRA ATC CODE: J7A9 Other specified single component
WHO ATC CODE: J07B-A Encephalitis vaccines
HIGHEST DEV. PHASE: Preclinical

COMPANY INFORMATION
ORIGINATOR: Nonindustrial source (United States)
PARENT: Nonindustrial source
OTHER: Southern Research Institute (CRO)

WORD COUNT: 422

L6 ANSWER 38 OF 40 COPYRIGHT 2001 Gale Group

AN 97:154502 NLDB
TI Salmonella typhimurium "Protective Immunity Against Salmonella typhimurium Elicited in Mice by Oral Vaccination with Phosphorylcholine **Encapsulated** in poly(DL-**lactide**-co-**glycolide**) **Microspheres**."
SO Vaccine Weekly, (21 Apr 1997) .
ISSN: 1074-2921.
PB Charles W Henderson
DT Newsletter
LA English
WC 387

L6 ANSWER 39 OF 40 PROMT COPYRIGHT 2001 Gale Group

ACCESSION NUMBER: 97:235638 PROMT
TITLE: Salmonella typhimurium "Protective Immunity Against Salmonella typhimurium Elicited in Mice by Oral Vaccination with Phosphorylcholine **Encapsulated** in poly(DL-**lactide**-co-**glycolide**) **Microspheres**."
SOURCE: Vaccine Weekly, (21 Apr 1997) pp. N/A.
ISSN: 1074-2921.
LANGUAGE: English
WORD COUNT: 387
FULL TEXT IS AVAILABLE IN THE ALL FORMAT

L6 ANSWER 40 OF 40 PROMT COPYRIGHT 2001 Gale Group

ACCESSION NUMBER: 93:600413 PROMT
TITLE: Microencapsulated Oral Influenza Vaccine Trials
SOURCE: Antiviral Agents Bulletin, (Mar 1993) pp. N/A.
ISSN: 0897-9871.

LANGUAGE: English
WORD COUNT: 665
FULL TEXT IS AVAILABLE IN THE ALL FORMAT

=> d l6 15, 16, 24, 25, 34, 35 kwic bib

L6 ANSWER 15 OF 40 USPATFULL
AB . . . at least most of the particles. Preferably the biodegradablepolymer is at least 5% by weight crystalline. Preferred biodegradable polymers are poly(L-**lactide**) (L.PLA) or copolymers or blends of L.PLA. The particles are especially useful for the immobilization of antigens or allergens for. . .

SUMM Although the influence of factors such as the dose, formulation and frequency of administration of **antigen** on the immune response is recognised, optimal delivery and presentation have not in general been established (Khan et al 1994). In conventional liquid dosing regimens, several small doses of **antigen** are more effective than a single inoculation or a few large doses in stimulating a protective immune response. It is. . . to stimulate a secondary response and that immunological unresponsiveness (tolerance) can be induced by both high and low doses of **antigen** and by frequent administration.

SUMM As well as protecting antigens, stimulating phagocytosis and activating lymphoid cells, some adjuvants function by retaining the **antigen** at the site of deposition. **Antigen** retention appears vital for repeated stimulation of the memory B-cell population and for maintaining antibody titres over long periods (Gray. . . Complete Adjuvant (FCA)/Freund's Incomplete Adjuvant (FIA), for example, is considered to arise from creation of a short-term `depot effect` involving **antigen** retention as a result of granuloma formation. Malarial **antigen** has been detected at the injection site 80 days post-administration when formulated with liposomes and **encapsulated** in alginate poly(L-lysine) microparticles (Cohen et al 1991) suggesting that this system also provides a `depot-type` vaccine for sustained retention. . .

SUMM The considerable research effort devoted to vaccine formulation has generated a multitude of strategies for optimising **antigen** release rates and achieving single dose delivery systems. Pulse release of **antigen** from biodegradable, biocompatible poly(**lactide** co-**glycolide**) [**PLG**] microparticles is considered advantageous for stimulating the conventional, multi-dose, schedule. However, most microparticulate delivery systems are considered to function on the principle of sustained, long term **antigen** release which presents a continuous trickle of **antigen** to the immune system to maintain proliferation of immune cells and antibody production. Raghuvanshi et al (1993) developed a single injection formulation for Tetanus toxoid (TT) based on this principle using **PLG** microparticles. The resultant immune response over 5 months in rats was comparable with the conventional 2-dose schedule of TT adsorbed. . .

SUMM The lower primary response observed with TT adsorbed to Alum was considered due to rapid **antigen** depletion resulting in reduced proliferation of immune cells.

SUMM The ability of small **antigen**-loaded **PLG** microparticles (<5 .mu.m in size) to function as potent **antigen** delivery systems after sub-cutaneous administration is considered to arise from 2 mechanisms: 1) efficient phagocytosis resulting in transport to the lymph nodes where efficient **antigen** processing and presentation to T-helper cells occurs and 2) controlled release of **antigen** from the microparticles. (Eldridge et al 1991 O'Hagan et al 1991). However, high immune responses have also been induced using. . . and transport to lymph nodes is not absolutely necessary for achieving high serum antibody titres. However, it is

recognised that **antigen**-containing fragments from large microparticles could be phagocytosed.

SUMM It is acknowledged that the higher immune response obtained when using **antigen**-loaded **PLG** microparticles could be attributed to an adjuvant effect rather than to slow release of **encapsulated** protein since antigens adsorbed onto microparticles have been shown to generate potent immune responses after subcutaneous (O'Hagan et al 1993.. . .

SUMM . . . be produced which are at least in part crystalline, and which have been found to give improvements in adsorption of **antigen**, retention of **antigen** in vitro and improvement in immune response to adsorbed antigens.

DRWD FIG. 1 is an electron micrograph of prior art spherical particles of poly(DL-**lactide**-co-**glycolide**) (**PLG**).

DETD A preferred polymer is poly(L-**lactide**) (L.PLA) which is semi-crystalline in nature. The molecular weight of the L.PLA polymer is preferably in the range 2,000 to. . .

DETD Suitable copolymers are copolymers of L.PLA and other poly(.alpha.-hydroxy acids) such as DL **lactide** or **glycolide** (eg. **PLG**), crystallisable copolymers of lactic acid and lactone, copolymers of L-**lactide** and poly(ethylene glycol) [PEG], copolymers of L-**lactide** and .alpha.-amino acids (polydepsipeptides), polyanhydrides, and polyorthoesters.

DETD Suitable blends of L.PLA with other polymers include other poly(.alpha.-hydroxy acids) such as poly(DL **lactide** co-**glycolide**), PEG, copolymers of polyethylene oxide and polypropylene oxide (PEO-PPO), polydepsipeptides, polyorthoesters, polyanhydrides, polyphosphazene and copolymers of acrylic and methacrylic acid. . .

DETD The active agent is preferably a vaccine, **antigen** or allergen or DNA.

DETD . . . and polysacchraides that are obtained from animal, plant, bacterial, viral and parasitic sources or produced by synthetic methods. The term **antigen** includes any material which will cause an antibody reaction of any sort when administered. Such antigens can be administered by. . .

DETD If the active agent is a peptide or protein drug, the lamellar particle, with the adsorbed active agent, is preferably **encapsulated** or enteric coated with polymer such as poly (D,L-**lactide** co-**glycolide**) (**PLG**) or a EUDRAGIT.TM. polymer, prior to oral administration.

DETD . . . The adsorption of active agents onto lamellar particles also avoids the disadvantages found with prior art microencapsulated vaccines based on **PLG**. These include avoidance of exposure to high shear forces and solvents and acid degradation products produced by **PLG** which may denature certain antigens. Furthermore, the lamellar particles have been found to have much greater retention of the **antigen** over long time periods in vitro. It is thought that the irregular lamellar form of the particles may function as. . .

DETD **Antigen** Adsorption

DETD . . . mg of particles (accurately weighed) produced by the method of Example 1 were incubated in an aqueous solution of an **antigen** overnight at room temperature with end-over end shaking (Voss mixer). The microparticles were centrifuged and washed once with distilled water. The supernatants were collected and analysed for **antigen** content using a BCA protein assay. A calibration curve was constructed from a series dilution of the respective **antigen** and the quantity of **antigen** adsorbed on the lamellar substrates was obtained by subtraction. The adsorbed amounts of the **antigen**, influenza virus, Tetanus toxoid and ovalbumin respectively are presented in Table 1.

DETD In Vitro **Antigen** Release from PLA Lamellar Substrate Particles

DETD 25-30 mg PLA lamellar particles with adsorbed **antigen** prepared

according to Example 3 were incubated in 2 ml PBS containing 0.02% Sodium azide at 37.degree. C. The release. . . sample tubes. This process was repeated at 3 day intervals up to 8 weeks. The release medium was analysed for **antigen** content using a BCA protein assay and the cumulative release amount of **antigen** (%) calculated. The retained amounts of Influenza virus, Tetanus toxoid and ovalbumin respectively are presented in Table 1.

DETD TABLE 1

Adsorption of antigens on lamellar poly(**lactide**) adjuvants

Antigen	% w/w adsorbed	Retained amount/time in vitro
Influenza virus	19.0	65% at 8 weeks
Tetanus toxoid	7.1	86% at 8 weeks
Ovalbumin		97%. . .

DETD . . . adsorbed to PLA lamellar substrate particles prepared by the methods of examples 1 and 3, and to prior art 75:25 **PLG microspheres** respectively. The adsorbed virus was allowed to stabilise for 14 weeks before commencing the immunogenicity study. The haemagglutinin (HA) content of the vaccines was calculated by estimating the amount of **antigen** remaining attached to the microparticles. The vaccine formulations were administered sub-cutaneously to groups of 20 Balb/C mice and test bleeds. . .

DETD 2. Inactivated virus, adsorbed to **PLG microspheres**, 15 .mu.g HA/0.1 ml

DETD . . . In addition, the response to virus adsorbed on PLA lamellar particles was almost five times that obtained using prior art **PLG microspheres** as a substrate for adsorption of influenza virus.

DETD Thermal transitions were recorded for PLA lamellar substrates and 75:25 **PLG** microspherical substrates using Differential Scanning Calorimetry. On heating at 20.degree. C./min from 20.degree. C. to 200.degree. C. a single melting. . .

DETD The 75:25 **PLG microspheres** showed a glass transition at 60.degree. C. on heating. After cooling and reheating the glass transition temperature was observed to have shifted to a slightly lower temperature of 57.degree. C. The amorphous nature of the 75:25 **PLG** copolymer results in an absence of melting or re-crystallisation peaks on thermal analysis.

DETD . . . The immune response was compared with that occurring in mice which received two doses of influenza virus adsorbed to 75:25 **PLG microspheres** and aqueous vaccine respectively. Microparticles without influenza virus were also administered as a control.

DETD . . . adsorbed lamellae vaccine were significantly better protected against virus challenged than those which received aqueous vaccine or virus adsorbed on **PLG microspheres**.

DETD . . . with virus loadings of 19% w/w and 13.1% w/w respectively. Influenza virus was also adsorbed onto lamellae prepared using a poly(L-**lactide**) polymer of Mw 90.600. An in vitro release study was conducted as described in Example 4. Cumulative release figures recorded. . .

DETD Alpar H. O., Almeida A. J. Identification of some of the physico-chemical characteristics of **microspheres** which influence the induction of the immune response following mucosal delivery. Eur. J. Pharm. Biopharm. 40, 198-202 (1994).

DETD Cohen S., Bernstein C., Hewes C., Chow M., Langer R. The pharmacokinetics of and humoral responses to **antigen** delivered by microencapsulated lipsomes. Proc. Natl. Acad. Sci. U.S.A. 88, 10440-10444 (1991).

DETD Eldridge J. H., Staas K., Meulbroek J. A., McGhee R., Tice T. R., Gilley

R. M. Biodegradable **microspheres** as a vaccine delivery system. Mol. Immunol. (1991), 28, 287-294.

DETD Gray D., Skarvall H. B-cell memory is short lived in the absence of **antigen**. Nature, (1988) 336, 70.

DETD . . . McGee P., Jeffery H., Davies M. C., Williams P., Davis S. S., Challacombe S. J. Biodegradable microparticles as controlled release **antigen** delivery systems. Immunology, 73, 239-242 (1991).

DETD . . . W. L., Crotts G. Importance of in vitro experimental conditions on protein release kinetics, stability and polymer degradation in protein **encapsulated** poly(DL lactic acid co-glycolic acid) **microspheres**. J. Controlled Rel., 33 (1995) 211-222.

DETD . . . A., Hem S. L., Lower J., Kreuter J. Comparison of 24 different adjuvants for inactivated HIV-2 split whole virus as **antigen** in mice. Induction of titres of binding antibodies and toxicity of the formulations. Vaccine, (1995) 13, 45-53.

CLM What is claimed is:
4. The composition of claim 1 wherein the biodegradable polymer is poly(L-**lactide**).

5. The composition of claim 1 wherein the biodegradable polymer is a copolymer of poly(L-**lactide**).

7. The composition of claim 6 wherein the **antigen** is selected from the group consisting of Tetanus toxoid and influenza virus.

AN 1999:163251 USPATFULL|
TI Polymeric lamellar substrate particles for drug delivery|
IN Coombes, Allan Gerald Arthur, Nottingham, United Kingdom
Davis, Stanley Stewart, Nottingham, United Kingdom
Major, Diane Lisa, London, United Kingdom
Wood, John Michael, Hertsfordshire, United Kingdom
PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)
PI US 6001395 19991214
WO 9702810 19970130
AI US 1998-983156 19980330 (8)
WO 1996-GB1695 19960715
19980330 PCT 371 date
19980330 PCT 102(e) date
PRAI GB 1995-14285 19950713
DT Utility|
FS Granted|
EXNAM Primary Examiner: Webman, Edward J.|
LREP Arnall Golden & Gregory, LLP|
CLMN Number of Claims: 18|
ECL Exemplary Claim: 1|
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)|
LN.CNT 793|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 16 OF 40 USPATFULL
AB A method, and compositions for use therein capable, of delivering a bioactive agent to an animal entailing the steps of **encapsulating** effective amounts of the agent in a biocompatible excipient to form microcapsules having a size less than approximately ten micrometers. . .

SUMM This invention relates to a method and a formulation for orally administering a bioactive agent **encapsulated** in one or more biocompatible polymer or copolymer excipients, preferably a biodegradable polymer or copolymer, affording microcapsules which due to. . .

SUMM The use of microencapsulation to protect sensitive bioactive agents from degradation has become well-known. Typically, a bioactive agent is **encapsulated** within any of a number of protective wall materials, usually polymeric in nature. The agent to be

encapsulated can be coated with a single wall of polymeric material (microcapsules), or can be homogeneously dispersed within a polymeric matrix (**microspheres**). (Hereafter, the term microcapsules refers to both microcapsules and **microspheres**). The amount of agent inside the microcapsule can be varied as desired, ranging from either a small amount to as. . .

SUMM . . . the body, and include such things as foreign protein or tissue. The immunologic response induced by the interaction of an **antigen** with the immune system may be either positive or negative with respect to the body's ability to mount an antibody or cell-mediated immune response to a subsequent reexposure to the **antigen**. Cell-mediated immune responses include responses such as the killing of foreign cells or tissues, "cell-mediated cytoxicity", and delayed-type hypersensitivity reactions. Antibodies belong to a class of proteins called immunoglobulins (Ig), which are produced in response to an **antigen**, and which combine specifically with the **antigen**. When an antibody and **antigen** combine, they form a complex. This complex may aid in the clearance of the **antigen** from the body, facilitate the killing of living antigens such as infectious agents and foreign tissues or cancers, and neutralize. . .

SUMM . . . secrete the antibody molecules. Studies by Heremans and Bazin measuring the development of IgA responses in mice orally immunized with **antigen** showed that a sequential appearance of **antigen** -specific IgA plasma cells occurred, first in mesenteric lymph nodes, later in the spleen, and finally in the lamina propria of. . . contribution of IgA antibody-forming cells to an immune response detected in extraintestinal lymphoid tissues of germ free mice exposed to **antigen** via the oral route. J. Immunol. 105:1049; 1970 and Crabbe, P. A., Nash, D. R., Bazin, H., Eyssen, H. and. . . S. J. and Babb, J. L. Selective induction of an immune response in human external secretions by ingestion of bacterial **antigen**. J. Clin. Invest. 61:731; 1978, Montgomery, P. C., Rosner, B. R. and Cohen, J. The secretory antibody response. Anti-DNP antibodies. . . 1007:165; 1978). It is apparent, therefore, that Peyer's patches are an enriched source of precursor IgA cells, which, subsequent to **antigen** sensitization, follow a circular migrational pathway and account for the expression of IgA at both the region of initial **antigen** exposure and at distant mucosal surfaces. This circular pattern provides a mucosal immune system by continually transporting sensitized B cells. . .

SUMM . . . immunization to induce protective antibodies. It is known that the ingestion of antigens by animals results in the appearance of **antigen**-specific sIgA antibodies in bronchial and nasal washings. For example, studies with human volunteers show that oral administration of influenza vaccine. . .

SUMM . . . a method of oral immunization which will effectively stimulate the immune system and overcome the problem of degradation of the **antigen** during its passage through the gastrointestinal tract to the Peyer's patch. There exists a more particular need for a method of targeting an **antigen** to the Peyer's patches and releasing that **antigen** once inside the body. There also exists a need for a method to immunize through other mucosal tissues of the body which overcomes the problems of degradation of the **antigen** and targets the delivery to the mucosally-associated lymphoid tissues. In addition, the need exists for the protection from degradation of. . .

SUMM It is an object of this invention to provide a method of orally administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the Peyer's patches, and thereby stimulating the mucosal immune system, without losing its effectiveness. . .

SUMM It is also an object of this invention to provide a method of orally administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the Peyer's patches, and

thereby stimulating the systemic immune system, without losing its effectiveness. . .

SUMM It is a further object of this invention to provide a method of administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the mucosally-associated lymphoid tissues, and thereby stimulating the mucosal immune system, without losing its. . .

SUMM It is a still further object of this invention to provide a method of administering an **antigen** to an animal which results in the **antigen** being taken up by the mucosally-associated lymphoid tissues, and thereby stimulating the systemic immune system, without losing its effectiveness as. . .

SUMM . . . is a still further object of this invention to provide a formulation consisting of a core bioactive ingredient and an **encapsulating** polymer or copolymer excipient which is biocompatible and preferably biodegradable as well, which can be utilized in the mucosal-administration methods. . .

SUMM . . . of this invention to provide an improved vaccine delivery system for the induction of immunity through the pulsatile release of **antigen** from a single administration of microencapsulated **antigen**.

SUMM . . . improved vaccine delivery system which both obviates the need for immunopotentiators and affords induction of immunity through pulsatile releases of **antigen** all from a single administration of microcapsulated **antigen**.

DRWD FIG. 1 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 .mu.m and 10-110 .mu.m 85:15 DL-**PLG** SEB toxoid-containing **microspheres**.

DRWD FIG. 3 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 pm 50:50 DL-**PLG**, 85:15 DL-**PLG**, and 100:0 L-PL3 SEB toxoid-containing microcapsules.

DRWD FIG. 4 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 .mu.m 50:50 DL-**PLG**, 100:0 L-**PLG**, and a mixture of 50:50 DL-**PLG** and 100:0 L-**PLG** SEB toxoid-containing microcapsules.

DETD . . . delivery of the antigens (trinitrophenyl keyhole limpet hemocyanin and a toxoid vaccine of staphylococcal enterotoxin B), and a drug (etretinate) **encapsulated** in 50:50 poly(DL-**lactide**-co-**glycolide**) to mice.

DETD It should be noted, however, that other polymers besides poly(DL-**lactide**-co-**glycolide**) may be used. Examples of such polymers include, but are not limited to, poly(**glycolide**), poly(DL-**lactide**-co-**glycolide**), copolyoxalates, polycaprolactone, poly(**lactide**-co-caprolactone), poly(esteramides), polyorthoesters and ploy(.beta.-hydroxybutyric acid), and polyanhydrides.

DETD TNP-KLH, a water-soluble **antigen**, was **encapsulated** in poly(DL-**lactide**-co-**glycolide**), a biocompatible, biodegradable polyester. The procedure used to prepare the microcapsules follows:

DETD First, a polymer solution was prepared by dissolving 0.5 g of 50:50 poly(DL-**lactide**-co-**glycolide**) in 4.0 g of methylene chloride. Next, 300 microliters of an aqueous solution of TNP-KLH (46 mg TNP-LKH/mL; after dialysis) was added to and homogeneously dispersed in the poly(DL-**lactide**-co-**glycolide**) solution by vortexing the mixture with a Vortex-Genie 2 (Scientific Industries, Inc., Bohemia, NY).

DETD The TNP-KLH content of the **antigen**-loaded microcapsules, that is, the core loading of the microcapsules, was determined by weighing out 10 mg of **antigen**-loaded microcapsules in a 12-mL centrifuge tube. Add 3.0 mL of methylene chloride to the tube and vortex to dissolve the poly(DL-**lactide**-co-**glycolide**). Next, add 3.0 mL of deionized water to the tube and vortex vigorously for 1 minute. Centrifuge the contents of. . .

DETD . . . of lymphoreticular tissue are located along the entire length of the small intestine and appendix. The targeted delivery of intact **antigen** directly into this tissue to achieve high local concentration is currently believed to be the most effective means of inducing. . .

DETD 85:15 Poly(DL-**lactide**-co-**glycolide**) Microcapsules

DETD . . . . a suspension in tap water via a gastric tube. The microcapsule wall material chosen for these studies consisted of 85:15 poly(DL-**lactide**-co-**glycolide**) due to its ability to resist significant bioerosion for a period of six weeks. At various times from 1 to. . .

DETD In additional experiments, tissue sections from Peyer's patches, mesenteric lymph node and spleen which contained absorbed 85:15 DL-**PLG** microcapsules were examined by histochemical and immunohistochemical techniques. Among other observations, these studies clearly showed that the microcapsules which were. . . by periodic acid Schiff's reagent (PAS) for intracellular carbohydrate, most probably glycogen, and for major histocompatibility complex (MHC) class II **antigen**. Further, the microcapsules observed in the mesenteric lymph nodes and in the spleen were universally found to have been carried there within these PAS and MHC class II positive cells. Thus, the **antigen** containing microcapsules have been internalized by **antigen**-presenting accessory cells (APC) in the Peyer's patches, and these APC have disseminated the **antigen**-microcapsules to other lymphoid tissues.

DETD . . . the size of the particles. Microcapsules <5 micrometers in diameter extravasate from the Peyer's patches within APC and release the **antigen** in lymphoid tissues which are inductive sites for systemic immune responses. In contrast, the microcapsules 5 to 10 micrometers in diameter remain in the Peyer's patches, also within APC, for extended time and release the **antigen** into this sIgA inductive site.

DETD . . . materials chosen for these studies consisted of polymers that varied in water uptake, biodegradation, and hydrophobicity. These polymers included polystyrene, poly(L-**lactide**), poly(DL-**lactide**), 50:50 poly(DL-**lactide**-co-**glycolide**), 85:15 poly(DL-**lactide**-co-**glycolide**), poly(hydroxybutyric acid), poly(methyl methacrylate), ethyl cellulose, cellulose acetate hydrogen phthalate, and cellulose triacetate. Microcapsules, prepared from 7 of the 10. . . hours after oral administration of a suspension containing 20 mg of microcapsules, as shown in Table 3. None of the **microspheres** were seen to penetrate into tissues other than the Peyer's patches. With one exception, ethyl cellulose, the efficiency of absorption. . . group of compounds [poly(styrene), poly(methyl methacrylate), poly(hydroxybutyrate)], while 200 to 1,000 microcapsules were observed with the relatively less hydrophobic polyesters [poly(L-**lactide**), poly(DL-**lactide**), 85:15 poly(DL-**lactide**-co-**glycolide**), 50:50 poly(DL-**lactide**-co-**glycolide**)]. As a class, the cellulosics were not absorbed.

DETD . . . Michalek, S. M. and McGhee, J. R. LPS regulation of the immune response: Suppression of immune response to orally-administered T-dependent **antigen**. J. Immunol. 127:1052; 1981).

DETD Research in our laboratories has shown that microencapsulation results in a profoundly heightened immune response to the incorporated **antigen** or vaccine in numerous experimental systems. An example is provided by the direct comparison of the level and isotype distribution. . . with either soluble or microencapsulated enterotoxoid. Groups of mice were administered various doses of the toxoid vaccine incorporated in 50:50 poly(DL-**lactide**-co-**glycolide**) microcapsules, or in soluble form, by intraperitoneal (IP) injection. On Days 10 and 20 following immunization, plasma samples were obtained. . .

DETD One hundred micrograms of enterotoxoid in **microspheres**

administered by SC injection at 4 sites along the backs of mice stimulated a peak IgG anti-toxin response equivalent to. . .

DETD When considering the mechanism through which 1-10 micrometer DL-**PLG microspheres** mediate a potentiated humoral immune response to the **encapsulated antigen**, three mechanisms must be considered as possibilities. First, the long term chronic release (depot), as compared to a bolus dose of nonencapsulated **antigen**, may play a role in immune enhancement. Second, our experiments have shown that **microspheres** in this size range are readily phagocytized by **antigen** processing and presenting cells. Therefore, targeted delivery of a comparatively large dose of nondegraded **antigen** directly to the cells responsible for the initiation of immune responses to T cell-dependent antigens must also be considered. Third,. . . Immunopotentiation by this latter mechanism has the characteristic that it is expressed when the adjuvant is administered concurrently with the **antigen**.

DETD In order to test whether **microspheres** possess any innate adjuvancy which is mediated through the ability of these particles to nonspecifically activate the immune system, the. . . of microencapsulated enterotoxoid was compared to that induced following the administration of an equal dose of enterotoxoid mixed with placebo **microspheres** containing no **antigen**. The various **antigen** forms were administered by IP injections into groups of 10 BALB/c mice and the plasma IgM and IgG enterotoxin-specific antibody. . .

DETD . . . IgG isotypes which was still increasing on day 30 after immunization. Co-administration of soluble enterotoxoid and a dose of placebo **microspheres** equal in weight, size and composition to those used to administer **encapsulated antigen** did not induce a plasma anti-toxin response which was significantly higher than that induced by soluble **antigen** alone. This result was not changed by the administration of the soluble **antigen** 1 day before or 1, 2 or 5 days after the placebo **microspheres**. Thus, these data indicate that the immunopotentiation expressed when **antigen** is administered within 1-10 micrometer DL-**PLG microspheres** is not a function of the ability of the **microspheres** to intrinsically activate the immune system. Rather, the data are consistent with either a depot effect, targeted delivery of the **antigen** to **antigen**-presenting accessory cells, or a combination of these two mechanisms.

DETD Retarding the **Antigen** Release Rate from 1-10 Micrometer Microcapsules Increases the Level of the Antibody Response and Delays the Time of the Peak. . .

DETD Four enterotoxoid containing microcapsule preparations with a variety of **antigen** release rates were compared for their ability to induce a plasma anti-toxin response following IP injection. The rate of **antigen** release by the microcapsules used in this study is a function of two mechanisms; diffusion through pores in the wall. . . of the rate at which the wall materials are hydrolyzed. However, these latter two lots differ in the ratio of **lactide** to **glycolide** composing the microcapsules, and the greater resistance of the 85:15 DL-**PLG** to hydrolysis results in a slower rate of enterotoxoid release.

DETD . . . 45 which were substantially higher (102,400) than those induced by either lot with early release. Further delaying the rate of **antigen** release through the use of an 85:15 ratio of **lactide** to **glycolide**, Batch #928-060-00 (0% release at 48 hours) delayed the peak antibody levels until days 45 and 60, but no further. . .

DETD These results are consistent with a delayed and sustained release of **antigen** stimulating a higher antibody response. However, certain aspects of the pattern of responses induced by these various **microspheres** indicate that a depot effect is not the only mechanism of immunopotentiation. The faster the initial release, the

lower the peak antibody titer. These results are consistent with a model in which the **antigen** released within the first 48 hours via diffusion through pores is no more effective than the administration of soluble **antigen**. Significant delay in the onset of release to allow time for phagocytosis of the **microspheres** by macrophages allows for the effective processing and presentation of the **antigen**, and the height of the resulting response is governed by the amount of **antigen** delivered into the presenting cells. However, delay of **antigen** release beyond the point where all the **antigen** is delivered into the presenting cells does not result in further potentiation of the response, it only delays the peak. . .

DETD It has been consistently observed that the size of the **microspheres** has a profound effect on the degree to which the antibody response is potentiated and the time at which it is initiated. These effects are best illustrated under conditions of a limiting **antigen** dose. Mice immunized subcutaneously with 10 .mu.g of SEB toxoid **encapsulated** in 1-10 .mu.m **microspheres** produced a more rapid, and a substantially more vigorous, IgG anti-toxin response than did mice immunized with the same dose of toxoid in 10-110 .mu.m **microspheres** as shown in FIG. 1. Groups of 5 mice were subcutaneously immunized with 10 .mu.g of SEB toxoid **encapsulated** in 1-10 .mu.m (85:15 DL-**PLG**; .065 wt % SEB toxoid) or 10-110 .mu.m (85:15 DL-**PLG**; 1.03 wt % SEB toxoid) **microspheres**. Plasma samples were obtained at 10 day intervals and the IgG anti-toxin titer determined by end-point titration in a RIA. . .

DETD A likely explanation for these effects involves the manner in which these different sizes of **microspheres** deliver **antigen** into the draining lymphatics. We have observed fluorescent DL-**PLG microspheres** of <10 .mu.m in diameter to be efficiently phagocytized and transported by macrophages into the draining lymph nodes. In contrast, larger **microspheres** (>10 .mu.m)remain localized at the site of injection. Taken together, these data suggest that the extremely strong adjuvant activity of <10 .mu.m **microspheres** is due to their efficient loading of **antigen** into accessory cells which direct the delivery of the microencapsulated **antigen** into the draining lymph nodes.

DETD . . . and a third injection is given to afford a tertiary response. Multiple injections are needed because repeated interaction of the **antigen** with immune system cells is required to stimulate a strong immunological response. After receiving the first injection of vaccine, a. . .

DETD The vaccine formulation that is injected into a patient may consist of an **antigen** in association with an adjuvant. For instance, an **antigen** can be bound to alum. During the first injection, the use of the **antigen**/adjuvant combination is important in that the adjuvant aids in the stimulation of an immune response. During the second and third injections, the administration of the **antigen** improves the immune response of the body to the **antigen**. The second and third administrations or subsequent administrations, however, do not necessarily require an adjuvant.

DETD Alza Corporation has described methods for the continuous release of an **antigen** and an immunopotentiator (adjuvant) to stimulate an immune response (U.S. Pat. No. 4,455,142). This invention differs from the Alza patent in at least two important manners. First, no immunopotentiator is required to increase the immune response, and second, the **antigen** is not continuously released from the delivery system.

DETD The present invention concerns the formulation of vaccine (**antigen**) into microcapsules (or **microspheres**) whereby the **antigen** is **encapsulated** in biodegradable polymers, such as poly(DL-**lactide**-co-**glycolide**). More specifically, different vaccine microcapsules are fabricated and

then mixed together such that a single injection of the vaccine capsule mixture improves the primary immune response and then delivers **antigen** in a pulsatile fashion at later time points to afford secondary, tertiary, and subsequent responses.

DETD . . . the small microcapsules are efficiently recognized and taken up by macrophages. The microcapsules inside of the macrophages then release the **antigen** which is subsequently processed and presented on the surface of the macrophage to give the primary response. The larger microcapsules,. . . preferably less than 250 micrometers, are made with different polymers so that as they biodegrade at different rates, they release **antigen** in a pulsatile fashion.

DETD Furthermore, the mixture of microcapsules may consist entirely of microcapsules sized less than 10 micrometers. **Microspheres** less than 10 micrometers in diameter are rapidly phagocytized by macrophages after administration. By using mixtures of **microspheres** less than 10 micrometers in diameter that have been prepared with polymers that have various **lactide/ glycolide** ratios, an immediate primary immunization as well as one or more discrete booster immunizations at the desired intervals (up to approximately eight months after administration) can be obtained. By mixing **microspheres** less than 10 micrometers in diameter (for the primary immunization) with **microspheres** greater than 10 micrometers in diameter, the time course possible for delivery of the discrete booster immunizations can be extended up to approximately 2 years. This longer time course is possible because the larger **microspheres** are not phagocytized and are therefore degraded at a slower rate than are the less than 10 micrometer **microspheres** .

DETD Using the present invention, the composition of the **antigen** microcapsules for the primary response is basically the same as the composition of the **antigen** microcapsules used for the secondary, tertiary, and subsequent responses. That is, the **antigen** is **encapsulated** with the same class of biodegradable polymers. The size and pulsatile release properties of the **antigen** microcapsules then maximizes the immune response to the **antigen**.

DETD The preferred biodegradable polymers are those whose biodegradation rates can be varied merely by altering their monomer ratio, for example, poly(DL-**lactide**-co-**glycolide**), so that **antigen** microcapsules used for the primary response will biodegrade faster than **antigen** microcapsules used for subsequent responses, affording pulsatile release of the **antigen** .

DETD . . . by controlling the size of the microcapsules of basically the same composition, one can maximize the immune response to an **antigen**. Also important is having small microcapsules (microcapsules less than 10 micrometers, preferably less than 5 micrometers, most preferably 1 to 5 micrometers) in the mixture of **antigen** microcapsules to maximize the primary response. The use of an immune enhancing delivery system, such as small microcapsules, becomes even. . .

DETD . . . response of mice immunized with a single administration of JE vaccine consisting of one part unencapsulated vaccine and two parts **encapsulated** vaccine. The JE microcapsules were >10 micrometers. The results of immunizing mice with JE vaccine by these two methods were. . .

DETD . . . 42 (standard schedule) and (4) mice which received 3.0 mg of JE vaccine (unencapsulated) and 3.0 mg of JE vaccine (**encapsulated**) on day 0 were studied. The untreated controls provide background virus neutralization titers against which immunized animals can be compared.. . . dose of JE vaccine on Day 0 provide background neutralization titers against which animals receiving unencapsulated vaccine in conjunction with **encapsulated** vaccine can be compared. This comparison provides evidence that the administration of

encapsulated vaccine augments the immunization potential of a single 3.0 mg dose of unencapsulated vaccine. The animals receiving 3 doses of unencapsulated vaccine provide controls against which the **encapsulated** vaccine group can be compared so as to document the ability of a single injection consisting of both nonencapsulated.and **encapsulated** vaccine to produce antiviral activity comparable to a standard three dose immunization schedule.

DETD . . . mean titer for this group decreased by greater than 50% from Day 40 to Day 77. All ten animals receiving **encapsulated** JE vaccine (Group 4) developed serum antiviral activity. The geometric mean titer for this group increased from Day 21 to. . . no significant difference in the average titer for these two groups in the Day 77 samples (p=0.75) indicating that the **encapsulated** vaccine group achieved comparable serum antiviral titers at Day 77. Unlike the 3 vaccine dose group (Group 3), the animals receiving **encapsulated** vaccine (Group 4) continued to demonstrate increases in serum virus neutralizing activity throughout the timepoints examined. In contrast to the standard vaccine treatment group, mice receiving **encapsulated** JE vaccine had a two-fold increase in the average serum neutralizing titer from Day 49 to Day 77. The Day. . . virus neutralizing titers similar to those produced by standard vaccine administration can be achieved by administering a single dose of **encapsulated** JE vaccine. Although the antiviral titers achieved with the excipient formulation used in this study did not increase as rapidly. . .

DETD . . . JE virus. The results of these assays, presented in Table 12, substantiate the findings described above. Although the animals receiving **encapsulated** vaccine did not reach peak,titers as rapidly as did the standard vaccine group, the **encapsulated** vaccine did induce comparable virus neutralizing antibody activity. Furthermore, the **encapsulated** vaccine maintained a higher antiviral titer over a longer period of time than did the standard vaccine. These results further. . .

DETD . . . and/or rate at which the incorporated material is released. In the case of vaccines this allows for scheduling of the **antigen** release in such a manner as to maximize the antibody response following a single administration. Among the possible release profiles. . .

DETD The possibility of using size as a mechanism to control vaccine release is based on the observation that **microspheres** <10 micrometers in diameter are phagocytized by macrophages and release **antigen** at a substantially accelerated rate relative to **microspheres** made of the same DL-**PLG** but which are too large to be phagocytized. The possibility of using size to achieve pulsed vaccine release was investigated by systemically (subcutaneously) injecting 100 micrograms of enterotoxoid to groups of mice either in 1-10 micrometer (50:50 DL-**PLG**; 1.51 wt % enterotoxoid), 20-50 micrometer (50:50 DL-**PLG**; 0.64 wt % enterotoxoid) or in a mixture of 1-10 micrometer and 20-50 micrometer microcapsules in which equal parts of. . .

DETD . . . through the co-administration of 1-10 and 20-50 micrometer enterotoxoid-containing microcapsules is consistent with a two phase (pulsed) release of the **antigen**. The first pulse results from the rapid ingestion and accelerated degradation of the 1-10 micrometer particles by tissue histiocytes, which results in a potentiated primary immune response due to the efficient loading of high concentrations of the **antigen** into these accessory cells, and most probably their activation. The second phase of **antigen** release is due to the biodegradation of the 20-50 micrometer microcapsules, which are too large to be ingested by phagocytic cells. This second pulse of **antigen** is released into a primed host and stimulates an anamnestic immune response. Thus, using the 50:50 DL-**PLG** copolymer, a single injection vaccine delivery system can be constructed which potentiates antibody responses (1-10 micrometer microcapsules), and which can. . .

DETD The hydrolysis rate of the DL-**PLG** copolymer can be changed by altering the **lactide**-to-**glycolide** ratio. This approach to the pulsed release of vaccine antigens was investigated in experiments in which groups of mice were subcutaneously immunized with 10 .mu.g of SEB toxoid in 1 to 10 micrometer **microspheres** formulated from DL-**PLG** with **lactide**-to-**glycolide** ratios of 50:50 or 85:15 DL-**PLG** or 100:0 L-**PLG**. Determination of the plasma IgG anti-toxin levels in these mice as a function of time demonstrated that these preparations of. . . at distinctly different times as shown in FIG. 3. Each preparation stimulated a peak IgG titer of 409,600, but the **microspheres** formulated of 50:50 and 85:15 DL-**PLG** and 100:0 L-**PLG** resulted in this level being attained on days 50, 130 and 230, respectively.

DETD The possibility of using a blend of 1 to 10 .mu.m **microspheres** with different DL-PLGs having different **lactide**/**glycolide** ratios to deliver discrete pulsed releases of **antigen** was investigated in a group of mice subcutaneously immunized in parallel. This blend consisted of 50:50 DL-**PLG** and 100:0 L-**PLG microspheres** in which each component contained 5 .mu.g of SEB toxoid. The plasma IgG anti-SEB toxin response induced by this mixture. . . FIG. 4. The first component of this response was coincident with that seen in mice which received only the 50:50 DL-**PLG microspheres**, while the second component coincided with the time at which the immune response was observed in mice receiving only the 100:0 L-**PLG microspheres**. The anamnestic character of the second phase indicates that distinct primary and secondary anti-SEB toxin responses have been induced.

DETD These data show that in a mixture of **microspheres** with differing **lactide/glycolide** ratios, the degradation rate of an individual **microsphere** is a function of its **lactide/glycolide** ratio and that it is independent of the degradation rate of the other **microspheres** in the mixture. This finding indicates that 1) the time at which any vaccine pulse can be delivered is continuously variable across the range of **lactide/glycolide** rations, 2) the pulsed vaccine release profiles of any combination of **microspheres** with differing **lactide/glycolide** ratios can be predicted with a high degree of certainty based on the behavior of the individual components, and 3) the delay in vaccine release possible with **microspheres** <10 .mu.m in diameter is up to approximately 8 months while the delay possible for **microspheres** >10 .mu.m is up to approximately 2 years, allowing for any number of discrete pulsatile vaccine releases over these time. . .

DETD . . . 5 micrometers, that will be engulfed by macrophages and obviate the need for immunopotentiators, as well as mixtures of free **antigen** for a primary response in combination with microcapsulated **antigen** in the form of microcapsules having a diameter of 10 micrometers or greater that release the **antigen** pulsatile to potentiate secondary and tertiary responses and provide immunization with a single administration. Also, a combination of small microcapsules. . .

DETD Orally-Administered **Microspheres** Containing TNP-KLH Induce Concurrent Circulating and Mucosal Antibody Responses to TNP.

DETD Microcapsules containing the haptenated protein **antigen** trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) were prepared using 50:50 DL-**PLG** as the excipient. These microcapsules were separated according to size and those in the range of 1 to 5 micrometers in diameter were selected for evaluation. These microcapsules contained 0.2% **antigen** by weight. Their ability to serve as an effective **antigen** delivery system when ingested was tested by administering 0.5 mL of a 10 mg/mL suspension (10 micrograms **antigen**) in bicarbonate-buffered sterile tap water via gastric

incubation on 4 consecutive days. For comparative purposes an additional group of mice. . .

DETD . . . administration of 30 micrograms of microencapsulated TNP-KLH in equal doses over 3 consecutive days resulted in the appearance of significant **antigen**-specific IgA antibodies in the secretions, and of all isotypes in the serum by Day 14 after immunization (see the last. . . These antibody levels were increased further on Day 28. In contrast, the oral administration of the same amount of unencapsulated **antigen** was ineffective at inducing specific antibodies of any isotype in any of the fluids tested.

DETD These results are noteworthy in several respects. First, significant **antigen**-specific IgA antibodies are induced in the serum and mucosal secretions, a response which is poor or absent following the commonly. . . mucosa; the portal of entry or site of pathology for a number of bacterial and viral pathogens. Secondly, the microencapsulated **antigen** preparation was an effective immunogen when orally administered, while the same amount of unencapsulated **antigen** was not. Thus, the microencapsulation resulted in a dramatic increase in efficacy, due to targeting of and increased uptake by. . . the absence of adjuvants is characterized by a peak in antibody levels in 7 to 14 days, the orally administered **antigen**-containing microcapsules induced responses were higher at Day 28 than Day 14. This indicates that bioerosion of the wall materials and release of the **antigen** is taking place over an extended period of time, and thus inducing a response of greater duration.

DETD . . . of mice were immunized with 100 micrograms of Staphylococcal enterotoxoid B in soluble form or within microcapsules with a 50:50 DL-**PLG** excipient. These mice were administered the soluble or microencapsulated toxoid via gastric tube on three occasions separated by 30 days,. . .

DETD These data demonstrate that microencapsulation allowed an immune response to take place against the **antigen** SEB toxoid following administration into the respiratory tract while the nonencapsulated **antigen** was ineffective. This response was observed both in the circulation and in the secretions bathing the respiratory tract. It should. . .

DETD In both man and animals, it has been shown that systemic immunization coupled with mucosal presentation of **antigen** is more effective than any other combination in promoting mucosal immune responses (Pierce, N. F. and Gowans, J. L. Cellular. . . either the IP, oral or IT routes. This was done to directly determine if a mixed immunization protocol utilizing microencapsulated **antigen** was advantageous with respect to the levels of sIgA induced.

DETD . . . antibody responses. Although the experiments reported here examine discrete priming and boosting steps which each required an administration of microencapsulated **antigen**, it will be possible to use the flexibility in controlled pulsatile release afforded by the microcapsule delivery system to design a single time of administration regimen which will stimulate maximum concurrent systemic and secretory immunity. As an example, microencapsulated **antigen** could be administered by both injection and ingestion during a single visit to a physician. By varying the **lactide** to **glycolide** ratio in the two doses, the systemically administered dose could be released within a few days to prime the immune. . .

DETD . . . of pharmaceuticals as well as antigens into the body. Etretinate, (All-E)-9-(4-methoxy-2,3,6,-trimethyl) phenyl-3, 7-dimethyl-2,4,8-nonatetraenoic acid, ethyl ester) was microencapsulated in 50:50 poly(DL-**lactide**-co-**glycolide**). The microcapsules were 0.5 to 4 micrometers in diameter and contained 37.2 wt % etretinate. These etretinate microcapsules, as well. . .

DETD TABLE 1

Penetration of Coumarin-6 85:15 DL-**PLG** **Microspheres** Into
and

Through the Peyer's Patches Following Oral Administration

Time (days)	Total number	Proportion of diameter (%) Small	Medium	Large	Proportion at location (%)
DETD	TABLE 2				

Migration of Coumarin-6 85:15 DL-**PLG Microspheres** Into and Through the Mesenteric Lymph Nodes Following Oral Administration

Time	Total number	Proportion of diameter (%) Small	Medium	Large	Proportion at location. . .
DETD	TABLE 3				

Targeted Absorption of 1- to 10-um **Microspheres** with Various Excipients by the Peyer's Patches of the Gut-Associated Lymphoid Tissues Following Oral Administration

Microsphere Excipient	Biodegradable	Absorption by the Peyer's patches
Poly(styrene)	No	Very Good
Poly(methyl methacrylate)	No	Very Good
Poly(hydroxybutyrate)	Yes	Very Good
Poly(DL-**lactide**)	Yes	Good
Poly(L-**lactide**)	Yes	Good
85:15 Poly(DL-**lactide**-co-**glycolide**)	Yes	Good
50:50 Poly(DL-**lactide**-co-**glycolide**)	Yes	Good
Cellulose acetate hydrogen phthalate	No	None
Cellulose triacetate	No	None
Ethyl cellulose	No	None
DETD	TABLE 9	

Microspheres Do not Possess Inherent Adjuvant Activity

Dose (.mu.g) of Toxoid	Form	Plasma Anti-Toxin Titer Day 10 IgM	Day 10 IgG	Day 20 IgM	Day 20 IgG	Day 30 IgM	Day 30 IgG
25	**Antigen** in Micro-spheres	6,400	6,400	400	12,800	800	25,600
25	Soluble **Antigen**	800	<50	200	800	100	<50
25	**Antigen** plus Placebo Micro-spheres	800	<50	200	<50	200	50
DETD	TABLE 10						

Systemic Anti-Toxin Response Induced by Parenteral Immunization .mu.m **Microspheres** Releasing **Antigen** at Various Rates

Dose (.mu.g) of Toxoid	Form	**Lactide/Glycolide** Ratio	**Antigen** release at 48 Hr	Plasma IgG Anti-Toxin Titer on Day 10	15	20	30	45	60
100	Soluble	--	--	<50	<50	<50	<50	<50	<50
100	**Microspheres**	50:50	60%	400	--	6,400	3,200	--	--
100	**Microspheres**	50:50	30%	400	--	12,800	6,400	--	--
100	**Microspheres**	50:50	10%	--	6,400	--	102,400	102,400	51,200
100	**Microspheres**	85:15	0%	--	3,200	--	51,200	102,400	102,400

DETD TABLE 14

Plasma IgM and IgG Anti-Toxin Levels on Day 20 Following Primary, Secondary, and Tertiary Oral Immunization with Soluble or Microencapsulated (50:50 DL-**PLG**) Staphylococcal Toxoid

Entero-toxoid dose (.mu.g) per immu-nization	Form	Plasma anti-toxin titer on day 20 following oral immunization: Primary IgM	Primary IgG	Secondary IgM. . .	Secondary IgG	Tertiary IgM. . .	Tertiary IgG

CLM What is claimed is:

. . . ophthalmically, or oral inhalationally administering an effective amount of microcapsules to said animal, wherein said microcapsules comprise said bioactive agent **encapsulated** in a biocompatible excipient and wherein said microcapsules are of a size of between approximately 1 micrometer and approximately 10. . . .

3. The method of claim 1, wherein said bioactive agent is a drug, nutrient, immunomodulator, lymphokine, monokine, cytokine, or **antigen**.

5. The method of claim 1, wherein said bioactive agent is an **antigen**.

11. The method of claim 1, wherein said biocompatible excipient is a poly(**lactide**-co-**glycolide**), poly(**lactide**), poly(**glycolide**), copolyoxalate, polycaprolactone, poly(**lactide**-co-caprolactone), poly(esteraminde), polyorthoester, poly(p-hydroxybutyric acid), polyanhydride, or a mixture thereof.

. . . ophthalmically, or oral inhalationally administering an effective amount of microcapsules to said animal, wherein said microcapsules comprise said bioactive agent **encapsulated** in a biocompatible excipient and wherein said microcapsules are of a size of less than approximately 10 micrometers.

AN 1999:99400 USPATFULL|
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissues and controlling their release|
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5942252 19990824
AI US 1995-469463 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility|
FS Granted|
EXNAM Primary Examiner: Azpuru, Carlos A.|
LREP Needle & Rosenberg|
CLMN Number of Claims: 23|
ECL Exemplary Claim: 1|
DRWN 4 Drawing Figure(s); 2 Drawing Page(s)|
LN.CNT 2060|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 24 OF 40 USPATFULL
AB A method, and compositions for use therein capable, of delivering a bioactive agent to an animal entailing the steps of **encapsulating** effective amounts of the agent in a. biocompatible excipient to form microcapsules having a size less than approximately ten micrometers. . .
SUMM B. Preparation of **Antigen**-Loaded Microcapsules.
SUMM EXAMPLE 2--85:15 Poly(DL-**lactide**-co-**glycolide**) Microcapsules.
SUMM EXAMPLE 2--Retarding the **Antigen** Release Rate from 1-10 Micrometer Microcapsules Increases the Level of the Antibody Response and Delays the Time of the Peak. . .
SUMM EXAMPLE 1--Orally Administered **Microspheres** Containing TNP-KLH Induce Concurrent Circulating and Mucosal Antibody Responses to TNP.
SUMM This invention relates to a method and a formulation for orally administering a bioactive agent **encapsulated** in one or more biocompatible polymer or copolymer excipients, preferably a biodegradable polymer or copolymer, affording microcapsules which due to. . .
SUMM The use of microencapsulation to protect sensitive bioactive agents from degradation has become well-known. Typically, a bioactive agent is **encapsulated** within any of a number of protective wall materials, usually polymeric in nature. The agent to be **encapsulated** can be coated with a single wall of polymeric material (microcapsules), or can be homogeneously dispersed within a polymeric matrix (**microspheres**). (Hereafter, the term microcapsules refers to both microcapsules and **microspheres**). The amount of agent inside the microcapsule can be varied as desired,

ranging from either a small amount to as. . .

SUMM . . . the body, and include such things as foreign protein or tissue. The immunologic response induced by the interaction of an **antigen** with the immune system may be either positive or negative with respect to the body's ability to mount an antibody or cell-mediated immune response to a subsequent reexposure to the **antigen**. Cell-mediated immune responses include responses such as the killing of foreign cells or tissues, "cell-mediated cytoxicity", and delayed-type hypersensitivity reactions. Antibodies belong to a class of proteins called immunoglobulins (Ig), which are produced in response to an **antigen**, and which combine specifically with the **antigen**. When an antibody and **antigen** combine, they form a complex. This complex may aid in the clearance of the **antigen** from the body, facilitate the killing of living antigens such as infectious agents and foreign tissues or cancers, and neutralize. . .

SUMM . . . secrete the antibody molecules. Studies by Heremans and Bazin measuring the development of IgA responses in mice orally immunized with **antigen** showed that a sequential appearance of **antigen** -specific IgA plasma cells occurred, first in mesenteric lymph nodes, later in the spleen, and finally in the lamina propria of. . . contribution of IgA antibody-forming cells to an immune response detected in extraintestinal lymphoid tissues of germ free mice exposed to **antigen** via the oral route. J. Immunol. 105:1049; 1970 and Crabbe, P. A., Nash, D. R., Bazin, H., Eyssen, H. and. . . S. J. and Babb, J. L. Selective induction of an immune response in human external secretions by ingestion of bacterial **antigen**. J. Clin. Invest. 61:731; 1978, Montgomery, P. C., Rosner, B. R. and Cohen, J. The secretory antibody response. Anti-DNP antibodies. . . 1007:165; 1978). It is apparent, therefore, that Peyer's patches are an enriched source of precursor IgA cells, which, subsequent to **antigen** sensitization, follow a circular migrational pathway and account for the expression of IgA at both the region of initial **antigen** exposure and at distant mucosal surfaces. This circular pattern provides a mucosal immune system by continually transporting sensitized B cells. . .

SUMM . . . immunization to induce protective antibodies. It is known that the ingestion of antigens by animals results in the appearance of **antigen**-specific sIgA antibodies in bronchial and nasal washings. For example, studies with human volunteers show that oral administration of influenza vaccine. . .

SUMM . . . a method of oral immunization which will effectively stimulate the immune system and overcome the problem of degradation of the **antigen** during its passage through the gastrointestinal tract to the Peyer's patch. There exists a more particular need for a method of targeting an **antigen** to the Peyer's patches and releasing that **antigen** once inside the body. There also exists a need for a method to immunize through other mucosal tissues of the body which overcomes the problems of degradation of the **antigen** and targets the delivery to the mucosally-associated lymphoid tissues. In addition, the need exists for the protection from degradation of. . .

SUMM It is an object of this invention to provide a method of orally administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the Peyer's patches, and thereby stimulating the mucosal immune system, without losing its effectiveness. . .

SUMM It is also an object of this invention to provide a method of orally administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the Peyer's patches, and thereby stimulating the systemic immune system, without losing its effectiveness. . .

SUMM It is a further object of this invention to provide a method of administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the mucosally-associated

lymphoid tissues, and thereby stimulating the mucosal immune system, without losing its. . .

SUMM It is a still further object of this invention to provide a method of administering an **antigen** to an animal which results in the **antigen** being taken up by the mucosally-associated lymphoid tissues, and thereby stimulating the systemic immune system, without losing its effectiveness as. . .

SUMM . . . is a still further object of this invention to provide a formulation consisting of a core bioactive ingredient and an **encapsulating** polymer or copolymer excipient which is biocompatible and preferably biodegradable as well, which can be utilized in the mucosal-administration methods. . .

SUMM . . . of this invention to provide an improved vaccine delivery system for the induction of immunity through the pulsatile release of **antigen** from a single administration of microencapsulated **antigen**.

SUMM . . . improved vaccine delivery system which both obviates the need for immunopotentiators and affords induction of immunity through pulsatile releases of **antigen** all from a single administration of microcapsulated **antigen**.

DRWD FIG. 1 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 .mu.m and 10-110 .mu.m 85:15 DL-**PLG** SEB toxoid-containing **microspheres**.

DRWD FIG. 3 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 .mu.m 50:50 DL-**PLG**, 85:15 DL-**PLG**, and 100:0 L-**PLG** SEB toxoid-containing microcapsules.

DRWD FIG. 4 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 .mu.m 50:50 DL-**PLG**, 100:0 L-**PLG**, and a mixture of 50:50 DL-**PLG** and 100:0 L-**PLG** SEB toxoid-containing microcapsules.

DETD . . . delivery of the antigens (trinitrophenyl keyhole limpet hemocyanin and a toxoid vaccine of staphylococcal enterotoxin B), and a drug (etretinate) **encapsulated** in 50:50 poly(DL-**lactide**-co-**glycolide**) to mice.

DETD It should be noted, however, that other polymers besides poly(DL-**lactide**-co-**glycolide**) may be used. Examples of such polymers include, but are not limited to, poly(**glycolide**), poly(DL-**lactide**-co-**glycolide**), copolyoxalates, polycaprolactone, poly(**lactide**-co-caprolactone), poly(esteramides), polyorthoesters and poly(.beta.-hydroxybutyric acid), and polyanhydrides.

DETD B. Preparation of **Antigen**-Loaded Microcapsules

DETD TNP-KLH, a water-soluble **antigen**, was **encapsulated** in poly(DL-**lactide**-co-**glycolide**), a biocompatible, biodegradable polyester. The procedure used to prepare the microcapsules follows:

DETD First, a polymer solution was prepared by dissolving 0.5g of 50:50 poly(DL-**lactide**-co-**glycolide**) in 4.0 g of methylene chloride. Next, 300 microliters of an aqueous solution of TNP-KLH (46 mg TNP-LKH/mL; after dialysis) was added to and homogeneously dispersed in the poly(DL-**lactide**-co-**glycolide**) solution by vortexing the mixture with a Vortex-Genie 2 (Scientific Industries, Inc., Bohemia, N.Y.).

DETD The TNP-KLH content of the **antigen**-loaded microcapsules, that is, the core loading of the microcapsules, was determined by weighing out 10 mg of **antigen**-loaded microcapsules in a 12-mL centrifuge tube. Add 3.0 mL of methylene chloride to the tube and vortex to dissolve the poly(DL-**lactide**-co-**glycolide**). Next, add 3.0 mL of deionized water to the tube and vortex vigorously for 1 minute. Centrifuge the contents of. . .

DETD . . . of lymphoreticular tissue are located along the entire length of the small intestine and appendix. The targeted delivery of intact **antigen** directly into this tissue to achieve high local

concentration is currently believed to be the most effective means of inducing. . .

DETD 85:15 Poly(DL-**lactide**-co-**glycolide**) Microcapsules

DETD . . . a suspension in tap water via a gastric tube. The microcapsule wall material chosen for these studies consisted of 85:15 poly(DL-**lactide**-co-**glycolide**) due to its ability to resist significant bioerosion for a period of six weeks. At various times from 1 to. . .

DETD In additional experiments, tissue sections from Peyer's patches, mesenteric lymph node and spleen which contained absorbed 85:15 DL-**PLG** microcapsules were examined by histochemical and immunohistochemical techniques. Among other observations, these studies clearly showed that the microcapsules which were. . . by periodic acid Schiff's reagent (PAS) for intracellular carbohydrate, most probably glycogen, and for major histocompatibility complex (MHC) class II **antigen**. Further, the microcapsules observed in the mesenteric lymph nodes and in the spleen were universally found to have been carried there within these PAS and MHC class II positive cells. Thus, the **antigen** containing microcapsules have been internalized by **antigen**-presenting accessory cells (APC) in the Peyer's patches, and these APC have disseminated the **antigen** -microcapsules to other lymphoid tissues.

DETD . . . the size of the particles. Microcapsules <5 micrometers in diameter extravasate from the Peyer's patches within APC and release the **antigen** in lymphoid tissues which are inductive sites for systemic immune responses. In contrast, the microcapsules 5 to 10 micrometers in diameter remain in the Peyer's patches, also within APC, for extended time and release the **antigen** into this sIgA inductive site.

DETD . . . materials chosen for these studies consisted of polymers that varied in water uptake, biodegradation, and hydrophobicity. These polymers included polystyrene, poly(L-**lactide**), poly(DL-**lactide**), 50:50 poly(DL-**lactide**-co-**glycolide**), 85:15 poly(DL-**lactide**-co-**glycolide**), poly(hydroxybutyric acid), poly(methyl methacrylate), ethyl cellulose, cellulose acetate hydrogen phthalate, and cellulose triacetate. Microcapsules, prepared from 7 of the 10. . . hours after oral administration of a suspension containing 20 mg of microcapsules, as shown in Table 3. None of the **microspheres** were seen to penetrate into tissues other than the Peyer's patches. With one exception, ethyl cellulose, the efficiency of absorption. . . group of compounds [poly(styrene), poly(methyl methacrylate), poly(hydroxybutyrate)], while 200 to 1,000 microcapsules were observed with the relatively less hydrophobic polyesters [poly(L-**lactide**), poly(DL-**lactide**), 85:15 poly(DL-**lactide**-co-**glycolide**), 50:50 poly(DL-**lactide**-co-**glycolide**)]. As a class, the cellulosics were not absorbed.

DETD . . . H., Michalek, S.M. and McGhee, J. R. LPS regulation of the immune response: Suppression of immune response to orally-administered T-dependent **antigen**. J. Immunol. 127:1052; 1981).

DETD Research in our laboratories has shown that microencapsulation results in a profoundly heightened immune response to the incorporated **antigen** or vaccine in numerous experimental systems. An example is provided by the direct comparison of the level and isotype distribution. . . with either soluble or microencapsulated enterotoxoid. Groups of mice were administered various doses of the toxoid vaccine incorporated in 50:50 poly(DL-**lactide**-co-**glycolide**) microcapsules, or in soluble form, by intraperitoneal (IP) injection. On Days 10 and 20 following immunization, plasma samples were obtained. . .

DETD One hundred micrograms of enterotoxoid in **microspheres** administered by SC injection at 4 sites along the backs of mice stimulated a peak IgG anti-toxin response equivalent to. . .

DETD When considering the mechanism through which 1-10 micrometer DL-

PLG microspheres mediate a potentiated humoral immune response to the **encapsulated antigen**, three mechanisms must be considered as possibilities. First, the long term chronic release (depot), as compared to a bolus dose of nonencapsulated **antigen**, may play a role in immune enhancement. Second, our experiments have shown that **microspheres** in this size range are readily phagocytized by **antigen** processing and presenting cells. Therefore, targeted delivery of a comparatively large dose of nondegraded **antigen** directly to the cells responsible for the initiation of immune responses to T cell-dependent antigens must also be considered. Third,. . . Immunopotentiation by this latter mechanism has the characteristic that it is expressed when the adjuvant is administered concurrently with the **antigen**.

DETD In order to test whether **microspheres** possess any innate adjuvancy which is mediated through the ability of these particles to nonspecifically activate the immune system, the. . . of microencapsulated enterotoxoid was compared to that induced following the administration of an equal dose of enterotoxoid mixed with placebo **microspheres** containing no **antigen**. The various **antigen** forms were administered by IP injections into groups of 10 BALB/c mice and the plasma IgM and IgG enterotoxin-specific antibody. . .

DETD . . . IgG isotypes which was still increasing on day 30 after immunization. Co-administration of soluble enterotoxoid and a dose of placebo **microspheres** equal in weight, size and composition to those used to administer **encapsulated antigen** did not induce a plasma anti-toxin response which was significantly higher than that induced by soluble **antigen** alone. This result was not changed by the administration of the soluble **antigen** 1 day before or 1, 2 or 5 days after the placebo **microspheres**. Thus, these data indicate that the immunopotentiation expressed when **antigen** is administered within 1-10 micrometer DL-**PLG** **microspheres** is not a function of the ability of the **microspheres** to intrinsically activate the immune system. Rather, the data are consistent with either a depot effect, targeted delivery of the **antigen** to **antigen**-presenting accessory cells, or a combination of these two mechanisms.

DETD Retarding the **Antigen** Release Rate from 1-10 Micrometer Microcapsules Increases the Level of the Antibody Response and Delays the Time of the Peak. . .

DETD Four enterotoxoid containing microcapsule preparations with a variety of **antigen** release rates were compared for their ability to induce a plasma anti-toxin response following IP injection. The rate of **antigen** release by the microcapsules used in this study is a function of two mechanisms; diffusion through pores in the wall. . . of the rate at which the wall materials are hydrolyzed. However, these latter two lots differ in the ratio of **lactide** to **glycolide** composing the microcapsules, and the greater resistance of the 85:15 DL-**PLG** to hydrolysis results in a slower rate of enterotoxoid release.

DETD . . . 45 which were substantially higher (102,400) than those induced by either lot with early release. Further delaying the rate of **antigen** release through the use of an 85:15 ratio of **lactide** to **glycolide**, Batch #928-060-00 (0% release at 48 hours) delayed the peak antibody levels until days 45 and 60, but no further. . .

DETD These results are consistent with a delayed and sustained release of **antigen** stimulating a higher antibody response. However, certain aspects of the pattern of responses induced by these various **microspheres** indicate that a depot effect is not the only mechanism of immunopotentiation. The faster the initial release, the lower the peak antibody titer. These results are consistent with a model in which the **antigen** released within the first 48 hours via diffusion through pores is no more effective than the administration of

soluble **antigen**. Significant delay in the onset of release to allow time for phagocytosis of the **microspheres** by macrophages allows for the effective processing and presentation of the **antigen**, and the height of the resulting response is governed by the amount of **antigen** delivered into the presenting cells. However, delay of **antigen** release beyond the point where all the **antigen** is delivered into the presenting cells does not result in further potentiation of the response, it only delays the peak. . .

DETD It has been consistently observed that the size of the **microspheres** has a profound effect on the degree to which the antibody response is potentiated and the time at which it is initiated. These effects are best illustrated under conditions of a limiting **antigen** dose. Mice immunized subcutaneously with 10 .mu.g of SEB toxoid **encapsulated** in 1-10 .mu.m **microspheres** produced a more rapid, and a substantially more vigorous, IgG anti-toxin response than did mice immunized with the same dose of toxoid in 10-110 .mu.m **microspheres** as shown in FIG. 1. Groups of 5 mice were subcutaneously immunized with 10 .mu.g of SEB toxoid **encapsulated** in 1-10 .mu.m (85:15 DL-**PLG**; 0.065 wt % SEB toxoid) or 10-110 .mu.m (85:15 DL-**PLG**; 1.03 wt % SEB toxoid) **microspheres**. Plasma samples were obtained at 10 day intervals and the IgG anti-toxin titer determined by end-point titration in a RIA. . .

DETD A likely explanation for these effects involves the manner in which these different sizes of **microspheres** deliver **antigen** into the draining lymphatics. We have observed fluorescent DL-**PLG microspheres** of <10 .mu.m in diameter to be efficiently phagocytized and transported by macrophages into the draining lymph nodes. In contrast, larger **microspheres** (>10 .mu.m)remain localized at the site of injection. Taken together, these data suggest that the extremely strong adjuvant activity of <10 .mu.m **microspheres** is due to their efficient loading of **antigen** into accessory cells which direct the delivery of the microencapsulated **antigen** into the draining lymph nodes.

DETD . . . and a third injection is given to afford a tertiary response. Multiple injections are needed because repeated interaction of the **antigen** with immune system cells is required to stimulate a strong immunological response. After receiving the first injection of vaccine, a. . .

DETD The vaccine formulation that is injected into a patient may consist of an **antigen** in association with an adjuvant. For instance, an **antigen** can be bound to alum. During the first injection, the use of the **antigen**/adjuvant combination is important in that the adjuvant aids in the stimulation of an immune response. During the second and third injections, the administration of the **antigen** improves the immune response of the body to the **antigen**. The second and third administrations or subsequent administrations, however, do not necessarily require an adjuvant.

DETD Alza Corporation has described methods for the continuous release of an **antigen** and an immunopotentiator (adjuvant) to stimulate an immune response (U.S. Pat. No. 4,455,142). This invention differs from the Alza patent in at least two important manners. First, no immunopotentiator is required to increase the immune response, and second, the **antigen** is not continuously released from the delivery system.

DETD The present invention concerns the formulation of vaccine (**antigen**) into microcapsules (or **microspheres**) whereby the **antigen** is **encapsulated** in biodegradable polymers, such as poly(DL-**lactide**-co-**glycolide**). More specifically, different vaccine microcapsules are fabricated and then mixed together such that a single injection of the vaccine capsule mixture improves the primary immune response and then delivers **antigen** in a pulsatile fashion at later time points to afford

secondary, tertiary, and subsequent responses.

DETD . . . the small microcapsules are efficiently recognized and taken up by macrophages. The microcapsules inside of the macrophages then release the **antigen** which is subsequently processed and presented on the surface of the macrophage to give the primary response. The larger microcapsules,. . . preferably less than 250 micrometers, are made with different polymers so that as they biodegrade at different rates, they release **antigen** in a pulsatile fashion.

DETD Furthermore, the mixture of microcapsules may consist entirely of microcapsules sized less than 10 micrometers. **Microspheres** less than 10 micrometers in diameter are rapidly phagocytized by macrophages after administration. By using mixtures of **microspheres** less than 10 micrometers in diameter that have been prepared with polymers that have various **lactide**/**glycolide** ratios, an immediate primary immunization as well as one or more discrete booster immunizations at the desired intervals (up to approximately eight months after administration) can be obtained. By mixing **microspheres** less than 10 micrometers in diameter (for the primary immunization) with **microspheres** greater than 10 micrometers in diameter, the time course possible for delivery of the discrete booster immunizations can be extended up to approximately 2 years. This longer time course is possible because the larger **microspheres** are not phagocytized and are therefore degraded at a slower rate than are the less than 10 micrometer **microspheres** .

DETD Using the present invention, the composition of the **antigen** microcapsules for the primary response is basically the same as the composition of the **antigen** microcapsules used for the secondary, tertiary, and subsequent responses. That is, the **antigen** is **encapsulated** with the same class of biodegradable polymers. The size and pulsatile release properties of the **antigen** microcapsules then maximizes the immune response to the **antigen**.

DETD The preferred biodegradable polymers are those whose biodegradation rates can be varied merely by altering their monomer ratio, for example, poly(DL-**lactide**-co-**glycolide**), so that **antigen** microcapsules used for the primary response will biodegrade faster than **antigen** microcapsules used for subsequent responses, affording pulsatile release of the **antigen** .

DETD . . . by controlling the size of the microcapsules of basically the same composition, one can maximize the immune response to an **antigen**. Also important is having small microcapsules (microcapsules less than 10 micrometers, preferably less than 5 micrometers, most preferably 1 to 5 micrometers) in the mixture of **antigen** microcapsules to maximize the primary response. The use of an immune enhancing delivery system, such as small microcapsules, becomes even. . .

DETD . . . response of mice immunized with a single administration of JE vaccine consisting of one part unencapsulated vaccine and two parts **encapsulated** vaccine. The JE microcapsules were >10 micrometers. The results of immunizing mice with JE vaccine by these two methods were. . .

DETD . . . 42 (standard schedule) and (4) mice which received 3.0 mg of JE vaccine (unencapsulated) and 3.0 mg of JE vaccine (**encapsulated**) on day 0 were studied. The untreated controls provide background virus neutralization titers against which immunized animals can be compared.. . . dose of JE vaccine on Day 0 provide background neutralization titers against which animals receiving unencapsulated vaccine in conjunction with **encapsulated** vaccine can be compared. This comparison provides evidence that the administration of **encapsulated** vaccine augments the immunization potential of a single 3.0 mg dose of unencapsulated vaccine. The animals receiving 3 doses of unencapsulated vaccine provide controls against which the

encapsulated vaccine group can be compared so as to document the ability of a single injection consisting of both nonencapsulated and **encapsulated** vaccine to produce antiviral activity comparable to a standard three dose immunization schedule.

DETD . . . mean titer for this group decreased by greater than 50% from Day 40 to Day 77. All ten animals receiving **encapsulated** JE vaccine (Group 4) developed serum antiviral activity. The geometric mean titer for this group increased from Day 21 to. . . no significant difference in the average titer for these two groups in the Day 77 samples (p=0.75) indicating that the **encapsulated** vaccine group achieved comparable serum antiviral titers at Day 77. Unlike the 3 vaccine dose group (Group 3), the animals receiving **encapsulated** vaccine (Group 4) continued to demonstrate increases in serum virus neutralizing activity throughout the timepoints examined. In contrast to the standard vaccine treatment group, mice receiving **encapsulated** JE vaccine had a two-fold increase in the average serum neutralizing titer from Day 49 to Day 77. The Day. . . virus neutralizing titers similar to those produced by standard vaccine administration can be achieved by administering a single dose of **encapsulated** JE vaccine. Although the antiviral titers achieved with the excipient formulation used in this study did not increase as rapidly. . .

DETD . . . JE virus. The results of these assays, presented in Table 12, substantiate the findings described above. Although the animals receiving **encapsulated** vaccine did not reach peak titers as rapidly as did the standard vaccine group, the **encapsulated** vaccine did induce comparable virus neutralizing antibody activity. Furthermore, the **encapsulated** vaccine maintained a higher antiviral titer over a longer period of time than did the standard vaccine. These results further. . .

DETD . . . and/or rate at which the incorporated material is released. In the case of vaccines this allows for scheduling of the **antigen** release in such a manner as to maximize the antibody response following a single administration. Among the possible release profiles. . .

DETD The possibility of using size as a mechanism to control vaccine release is based on the observation that **microspheres** <10 micrometers in diameter are phagocytized by macrophages and release **antigen** at a substantially accelerated rate relative to **microspheres** made of the same DL-**PLG** but which are too large to be phagocytized. The possibility of using size to achieve pulsed vaccine release was investigated by systemically (subcutaneously) injecting 100 micrograms of enterotoxoid to groups of mice either in 1-10 micrometer (50:50 DL-**PLG**; 1.51 wt % enterotoxoid), 20-50 micrometer (50:50 DL-**PLG**; 0.64 wt % enterotoxoid) or in a mixture of 1-10 micrometer and 20-50 micrometer microcapsules in which equal parts of. . .

DETD . . . through the co-administration of 1-10 and 20-50 micrometer enterotoxoid-containing microcapsules is consistent with a two phase (pulsed) release of the **antigen**. The first pulse results from the rapid ingestion and accelerated degradation of the 1-10 micrometer particles by tissue histiocytes, which results in a potentiated primary immune response due to the efficient loading of high concentrations of the **antigen** into these accessory cells, and most probably their activation. The second phase of **antigen** release is due to the biodegradation of the 20-50 micrometer microcapsules, which are too large to be ingested by phagocytic cells. This second pulse of **antigen** is released into a primed host and stimulates an anamnestic immune response. Thus, using the 50:50 DL-**PLG** copolymer, a single injection vaccine delivery system can be constructed which potentiates antibody responses (1-10 micrometer microcapsules), and which can. . .

DETD The hydrolysis rate of the DL-**PLG** copolymer can be changed by altering the **lactide**-to-**glycolide** ratio. This approach to the pulsed release of vaccine antigens was investigated in

experiments in which groups of mice were subcutaneously immunized with 10 .mu.g of SEB toxoid in 1 to 10 micrometer **microspheres** formulated from DL-**PLG** with **lactide**-to-**glycolide** ratios of 50:50 or 85:15 DL-**PLG** or 100:0 L-**PLG**. Determination of the plasma IgG anti-toxin levels in these mice as a function of time demonstrated that these preparations of. . . at distinctly different times as shown in FIG. 3. Each preparation stimulated a peak IgG titer of 409,600, but the **microspheres** formulated of 50:50 and 85:15 DL-**PLG** and 100:0 L-**PLG** resulted in this level being attained on days 50, 130 and 230, respectively. The possibility of using a blend of 1 to 10 .mu.m **microspheres** with different DL-PLGs having different **lactide/glycolide** ratios to deliver discrete pulsed releases of **antigen** was investigated in a group of mice subcutaneously immunized in parallel. This blend consisted of 50:50 DL-**PLG** and 100:0 L-**PLG microspheres** in which each component contained 5 .mu.g of SEB toxoid. The plasma IgG anti-SEB toxin response induced by this mixture. . . FIG. 4. The first component of this response was coincident with that seen in mice which received only the 50:50 DL-**PLG microspheres**, while the second component coincided with the time at which the immune response was observed in mice receiving only the 100:0 L-**PLG microspheres**. The anamnestic character of the second phase indicates that distinct primary and secondary anti-SEB toxin responses have been induced.

DETD These data show that in a mixture of **microspheres** with differing **lactide/glycolide** ratios, the degradation rate of an individual **microsphere** is a function of its **lactide/glycolide** ratio and that it is independent of the degradation rate of the other **microspheres** in the mixture. This finding indicates that 1) the time at which any vaccine pulse can be delivered is continuously variable across the range of **lactide/glycolide** rations, 2) the pulsed vaccine release profiles of any combination of **microspheres** with differing **lactide/glycolide** ratios can be predicted with a high degree of certainty based on the behavior of the individual components, and 3) the delay in vaccine release possible with **microspheres** <10 .mu.m in diameter is up to approximately 8 months while the delay possible for **microspheres** >10 .mu.m is up to approximately 2 years, allowing for any number of discrete pulsatile vaccine releases over these time. . .

DETD . . . 5 micrometers, that will be engulfed by macrophages and obviate the need for immunopotentiators, as well as mixtures of free **antigen** for a primary response in combination with microcapsulated **antigen** in the form of microcapsules having a diameter of 10 micrometers or greater that release the **antigen** pulsatile to potentiate secondary and tertiary responses and provide immunization with a single administration. Also, a combination of small microcapsules. . .

DETD Orally-Administered **Microspheres** Containing TNP-KLH Induce Concurrent Circulating and Mucosal Antibody Responses to TNP

DETD Microcapsules containing the haptenated protein **antigen** trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) were prepared using 50:50 DL-**PLG** as the excipient. These microcapsules were separated according to size and those in the range of 1 to 5 micrometers in diameter were selected for evaluation. These microcapsules contained 0.2% **antigen** by weight. Their ability to serve as an effective **antigen** delivery system when ingested was tested by administering 0.5 mL of a 10 mg/mL suspension (10 micrograms **antigen**) in bicarbonate-buffered sterile tap water via gastric incubation on 4 consecutive days. For comparative purposes an additional group of mice. . .

DETD . . . administration of 30 micrograms of microencapsulated TNP-KLH in equal doses over 3 consecutive days resulted in the appearance of

significant **antigen**-specific IgA antibodies in the secretions, and of all isotypes in the serum by Day 14 after immunization (see the last. . . These antibody levels were increased further on Day 28. In contrast, the oral administration of the same amount of unencapsulated **antigen** was ineffective at inducing specific antibodies of any isotype in any of the fluids tested.

DETD These results are noteworthy in several respects. First, significant **antigen**-specific IgA antibodies are induced in the serum and mucosal secretions, a response which is poor or absent following the commonly. . . mucosa; the portal of entry or site of pathology for a number of bacterial and viral pathogens. Secondly, the microencapsulated **antigen** preparation was an effective immunogen when orally administered, while the same amount of unencapsulated **antigen** was not. Thus, the microencapsulation resulted in a dramatic increase in efficacy, due to targeting of and increased uptake by. . . the absence of adjuvants is characterized by a peak in antibody levels in 7 to 14 days, the orally administered **antigen**-containing microcapsules induced responses were higher at Day 28 than Day 14. This indicates that bioerosion of the wall materials and release of the **antigen** is taking place over an extended period of time, and thus inducing a response of greater duration.

DETD . . . of mice were immunized with 100 micrograms of Staphylococcal enterotoxoid B in soluble form or within microcapsules with a 50:50 DL-**PLG** excipient. These mice were administered the soluble or microencapsulated toxoid via gastric tube on three occasions separated by 30 days,. . .

DETD These data demonstrate that microencapsulation allowed an immune response to take place against the **antigen** SEB toxoid following administration into the respiratory tract while the nonencapsulated **antigen** was ineffective. This response was observed both in the circulation and in the secretions bathing the respiratory tract. It should. . .

DETD In both man and animals, it has been shown that systemic immunization coupled with mucosal presentation of **antigen** is more effective than any other combination in promoting mucosal immune responses (Pierce, N. F. and Gowans, J. L. Cellular. . . either the IP, oral or IT routes. This was done to directly determine if a mixed immunization protocol utilizing microencapsulated **antigen** was advantageous with respect to the levels of sIgA induced.

DETD . . . antibody responses. Although the experiments reported here examine discrete priming and boosting steps which each required an administration of microencapsulated **antigen**, it will be possible to use the flexibility in controlled pulsatile release afforded by the microcapsule delivery system to design a single time of administration regimen which will stimulate maximum concurrent systemic and secretory immunity. As an example, microencapsulated **antigen** could be administered by both injection and ingestion during a single visit to a physician. By varying the **lactide** to **glycolide** ratio in the two doses, the systemically administered dose could be released within a few days to prime the immune. . .

DETD . . . of pharmaceuticals as well as antigens into the body. Etretinate, (A11-E)-9-(4-methoxy-2,3,6,-trimethyl) phenyl-3, 7-dimethyl-2,4,8-nonatetraenoic acid, ethyl ester) was microencapsulated in 50:50 poly(DL-**lactide**-co-**glycolide**). The microcapsules were 0.5 to 4 micrometers in diameter and contained 37.2 wt % etretinate. These etretinate microcapsules, as well. . .

DETD TABLE 1

Penetration of Coumarin-6 85:15 DL-**PLG** **Microspheres** Into and Through the Peyer's Patches Following Oral Administration

Time	Total number	Proportion of diameter (%) Small	Medium	Large	Proporption at location (%)

(days). . .

DETD TABLE 2

Nigration of Coumarin-6 85:15 DL-**PLG Microspheres** Into and Through the Mesenteric Lymph Nodes Following Oral Administration

Time	Total number	Proportion of diameter (%) Small	Medium	Large	Proporption at location. . .

DETD TABLE 3

Targeted Absorption of 1- to 10-um **Microspheres** with Various. Excipients by the Peyer's Patches of the Gut-Associated Lymphoid Tissues Following Oral Admnistration

Microsphere Excipient	Biodegradable	Absorption by the Peyer's patches
Poly (styrene)	No	Very Good
Poly (methyl methacrylate)	No	Very Good
Poly(hydroxybutyrate)	Yes	Very Good
Poly(DL-**lactide**)	Yes	Good
Poly(L-**lactide**)	Yes	Good
85:15 Poly(DL-**lactide**-co-**glycolide**)	Yes	Good
50:50 Poly(DL-**lactide**-co-**glycolide**)	Yes	Good
Cellulose acetate hydrogen phthalate	No	None
Cellulose triacetate	No	None
Ethyl cellulose	No	None

DETD TABLE 9

Microspheres Do not Possess Inherent Adjuvant Activity

Dose (.mu.g) of Toxiod Form	Plasma Anti-Toxin Titer Day 10 IgM	Day 10 IgG	Day 20 IgM	Day 20 IgG	Day 30 IgM	Day 30 IgG
25 **Antigen** in	6,400	6,400	400	12,800	800	25,600
Microspheres 25 Soluble **Antigen**	800	<50	200	800	100	<50
25 **Antigen** plus	800	<50	200	<50	200	50
Placebo Micropheres						

DETD TABLE 10

Systemic Anti-Toxin Response Induced by Parenteral Immunization .mu.m **Microspheres** Releasing **Antigen** at Various Rates

Dose (.mu.g) of Toxiod	Form	**Lactide/ Glycolide** Ratio	**Antigen** release at 48 Hr	Plasma IgG Anti-Toxin Titer on Day 10	15	20	30	45	60
100	Soluble	--	--	<50	<50	<50	<50	<50	<50
100	**Microspheres**	50:50	60%	400	--	6,400	3,200	--	--
100	**Microspheres**	50:50	30%	400	--	12,800	6,400	--	--
100	**Microspheres**	50:50	10%	--	6,400	--	102,400	102,400	51,200
100	**Microspheres**	85:15	0%	--	3,200	--	51,200	102,400	102,400

DETD TABLE 14

Plasma IgM and IgG Anti-Toxin Levels on Day 20 Following Primary, Secondary, and Tertiary Oral Immunization with Soluble or Microencapsulated (50:50 DL-**PLG**) Staphylococcal Toxoid

Enter-otoxoid does (.mu.g) per immuni-zation	Form	Plasma anti-toxin titer on day 20 following oral immunization Primary IgM	Primary IgG	Secondary IgM	Secondary IgG	Tertiary IgM	Tertiary IgG
100	**Microspheres**	80	1,280	320	5,120	1,280	

40,960

100 Soluble <20 <20

80 <20

640

<20

CLM What is claimed is:

. . . first biocompatible microcapsules having a size of between approximately 1 micrometer and approximately 10 micrometers and containing a bioactive agent **encapsulated** in a first biocompatible excipient and administering second biocompatible microcapsules containing a bioactive agent **encapsulated** in a second biocompatible excipient, said first microcapsules providing a primary immune response and said second microcapsules releasing said agent. . .

24. The method of claim 1, wherein said bioactive agents are independently an immunomodulator, lymphokine, monokine, cytokine, or **antigen**.

26. The method of claim 25, wherein said antigens are independently an allergen, viral **antigen**, bacterial **antigen**, protozoan **antigen**, or a fungal **antigen**.

27. The method of claim 25, wherein said antigens are independently an influenzae **antigen**, Staphylococcus **antigen**, respiratory syncytial **antigen**, parainfluenza virus **antigen**, Hemophilus influenza **antigen**, Bordetella pertussis **antigen**, Neisseria gonorrhoea **antigen**, Streptococcus pneumoniae **antigen**, Plasmodium falciparum **antigen**, helminthic pathogen **antigen**, or an **antigen** to vaccinate against allergies.

41. The method of claim 1, wherein said first and said second biocompatible excipients are independently a poly(**lactide**-co-**glycolide**), polylactide), poly(**glycolide**), copolyoxalate, polycaprolactone, poly(**lactide** -co-caprolactone), poly(esteramide), polyorthoester, poly(.beta.-hydroxybutyric acid), polyanhydride, or a mixture thereof.

42. The method of claim 1, where sin aid first biocompatible excipient comprises poly(**lactide**-co-**glycolide**) having a first monomer ratio and said second biocompatible excipient comprises poly(**lactide**-co-**glycolide**) having a second monomer ratio or poly(**lactide**), said first and said second monomer ratios being chosen so as to provide different biodegradation rates for said first and. . .

. . . effective amounts of first biocompatible microcapsules having a size of less than approximately 10 micrometers and containing a bioactive agent **encapsulated** in a first biocompatible excipient and administering second biocompatible microcapsules containing a bioactive agent **encapsulated** in a second biocompatible excipient, said first microcapsules providing a primary immune response and said second microcapsules releasing said agent. . .

. . . first biocompatible microcapsules having a size of between approximately 1 micrometer and approximately 10 micrometers and containing a bioactive agent **encapsulated** in a first biocompatible excipient, said first microcapsules releasing said bioactive agent contained in said first microcapsules in a pulsed. . .

. . . said priming is by second microcapsules having a size of greater than approximately 10 micrometers and containing said bioactive agent **encapsulated** in a second biocompatible excipient.

. . . said priming is by second microcapsules having a size of greater than approximately 10 micrometers and containing said bioactive agent

encapsulated in a second biocompatible excipient.

. . . said priming is by second microcapsules having a size of greater than approximately 10 micrometers and containing said bioactive agent **encapsulated** in a second biocompatible excipient.

54. The method of claim 45, wherein said bioactive agents are independently an immunomodulator, lymphokine, monokine, cytokine, or **antigen**.

56. The method of claim 55, wherein said antigens are independently an allergen, viral **antigen**, bacterial **antigen**, protozoan **antigen**, or a fungal **antigen**.

57. The method of claim 55, wherein said antigens are independently an influenzae **antigen**, Staphylococcus **antigen**, respiratory syncytial **antigen**, parainfluenza virus **antigen**, Hemophilus influenza **antigen**, Bordetella pertussis **antigen**, Neisseria gonorrhoea **antigen**, Streptococcus pneumoniae **antigen**, Plasmodium falciparum **antigen**, helminthic pathogen **antigen**, or an **antigen** to vaccinate against allergies.

72. The method of claim 45, wherein said first biocompatible excipient is a poly(**lactide**-co-**glycolide**), poly(**lactide**), poly(**glycolide**), copolyoxalate, polycaprolactone, poly(**lactide**-co-caprolactone), poly(esteramide), polyorthoester, poly(.beta.-hydroxybutyric acid), polyanhydride, or a mixture thereof.

. . . effective amount of first biocompatible microcapsules having a size of less than approximately 10 micrometers and containing a bioactive agent **encapsulated** in a first biocompatible excipient, said first microcapsules releasing said bioactive agent contained in said first microcapsules in a pulsed. . .

AN 1998:124217 USPATFULL|
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissues and controlling their release|
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5820883 19981013
AI US 1995-468064 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility|
FS Granted|
EXNAM Primary Examiner: Lovering, Richard D.|
LREP Needle & Rosenberg, P.C.|
CLMN Number of Claims: 74|
ECL Exemplary Claim: 1|
DRWN 4 Drawing Figure(s); 2 Drawing Page(s)|
LN.CNT 2355|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 25 OF 40 USPATFULL

AB A method, and compositions for use therein capable, of delivering a bioactive agent to an animal entailing the steps of **encapsulating** effective amounts of the agent in a biocompatible excipient to form microcapsules having a size less than approximately ten micrometers. . .

SUMM B. Preparation of **Antigen**-Loaded Microcapsules.

SUMM EXAMPLE 2--85:15 Poly(DL-**lactide**-co-**glycolide**) Microcapsules.

SUMM EXAMPLE 2--Retarding the **Antigen** Release Rate from 1-10 Micrometer Microcapsules Increases the Level of the Antibody Response and Delays the Time of the Peak. . .

SUMM EXAMPLE 1--Orally Administered **Microspheres** Containing TNP-KLH Induce Concurrent Circulating and Mucosal Antibody Responses to TNP.

SUMM This invention relates to a method and a formulation for orally administering a bioactive agent **encapsulated** in one or more biocompatible polymer or copolymer excipients, preferably a biodegradable polymer or copolymer, affording microcapsules which due to. . .

SUMM The use of microencapsulation to protect sensitive bioactive agents from degradation has become well-known. Typically, a bioactive agent is **encapsulated** within any of a number of protective wall materials, usually polymeric in nature. The agent to be **encapsulated** can be coated with a single wall of polymeric material (microcapsules), or can be homogeneously dispersed within a polymeric matrix (**microspheres**). (Hereafter, the term microcapsules refers to both microcapsules and **microspheres**). The amount of agent inside the microcapsule can be varied as desired, ranging from either a small amount to as. . .

SUMM . . . the body, and include such things as foreign protein or tissue. The immunologic response induced by the interaction of an **antigen** with the immune system may be either positive or negative with respect to the body's ability to mount an antibody or cell-mediated immune response to a subsequent reexposure to the **antigen**. Cell-mediated immune responses include responses such as the killing of foreign cells or tissues, "cell-mediated cytoxicity", and delayed-type hypersensitivity reactions. Antibodies belong to a class of proteins called immunoglobulins (Ig), which are produced in response to an **antigen**, and which combine specifically with the **antigen**. When an antibody and **antigen** combine, they form a complex. This complex may aid in the clearance of the **antigen** from the body, facilitate the killing of living antigens such as infectious agents and foreign tissues or cancers, and neutralize. . .

SUMM . . . secrete the antibody molecules. Studies by Heremans and Bazin measuring the development of IgA responses in mice orally immunized with **antigen** showed that a sequential appearance of **antigen** -specific IgA plasma cells occurred, first in mesenteric lymph nodes, later in the spleen, and finally in the lamina propria of. . . contribution of IgA antibody-forming cells to an immune response detected in extraintestinal lymphoid tissues of germ free mice exposed to **antigen** via the oral route. J. Immunol. 105:1049; 1970 and Crabbe, P. A., Nash, D. R., Bazin, H., Eyssen, H. and. . . S. J. and Babb, J. L. Selective induction of an immune response in human external secretions by ingestion of bacterial **antigen**. J. Clin. Invest. 61:731; 1978, Montgomery, P. C., Rosner, B. R. and Cohen, J. The secretory antibody response. Anti-DNP antibodies. . . 1007:165; 1978). It is apparent, therefore, that Peyer's patches are an enriched source of precursor IgA cells, which, subsequent to **antigen** sensitization, follow a circular migrational pathway and account for the expression of IgA at both the region of initial **antigen** exposure and at distant mucosal surfaces. This circular pattern provides a mucosal immune system by continually transporting sensitized B cells.

. .

SUMM . . . immunization to induce protective antibodies. It is known that the ingestion of antigens by animals results in the appearance of **antigen**-specific sIgA antibodies in bronchial and nasal washings. For example, studies with human volunteers show that oral administration of influenza vaccine. . .

SUMM . . . a method of oral immunization which will effectively stimulate the immune system and overcome the problem of degradation of the **antigen** during its passage through the gastrointestinal tract to the Peyer's patch. There exists a more particular need for a method of targeting an **antigen** to the Peyer's patches and releasing that **antigen** once inside the body. There also exists a need for a method to immunize through other mucosal tissues of the body which overcomes the problems of degradation of the **antigen** and targets the delivery to the mucosally-associated lymphoid tissues. In addition, the need exists for the protection from degradation of. . .

SUMM It is an object of this invention to provide a method of orally administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the Peyer's patches, and thereby stimulating the mucosal immune system, without losing its effectiveness. . .

SUMM It is also an object of this invention to provide a method of orally administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the Peyer's patches, and thereby stimulating the systemic immune system, without losing its effectiveness. . .

SUMM It is a further object of this invention to provide a method of administering an **antigen** to an animal which results in the **antigen** reaching and being taken up by the mucosally-associated lymphoid tissues, and thereby stimulating the mucosal immune system, without losing its. . .

SUMM It is a still further object of this invention to provide a method of administering an **antigen** to an animal which results in the **antigen** being taken up by the mucosally-associated lymphoid tissues, and thereby stimulating the systemic immune system, without losing its effectiveness as. . .

SUMM . . . is a still further object of this invention to provide a formulation consisting of a core bioactive ingredient and an **encapsulating** polymer or copolymer excipient which is biocompatible and preferably biodegradable as well, which can be utilized in the mucosal-administration methods. . .

SUMM . . . of this invention to provide an improved vaccine delivery system for the induction of immunity through the pulsatile release of **antigen** from a single administration of microencapsulated **antigen**.

SUMM . . . improved vaccine delivery system which both obviates the need for immunopotentiators and affords induction of immunity through pulsatile releases of **antigen** all from a single administration of microcapsulated **antigen**.

DRWD FIG. 1 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 .mu.m and 10-110 .mu.m 85:15 DL-**PLG** SEB toxoid-containing **microspheres**.

DRWD FIG. 3 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 .mu.m 50:50 DL-**PLG**, 85:15 DL-**PLG**, and 100:0 L-**PLG** SEB toxoid-containing microcapsules.

DRWD FIG. 4 represents the plasma IgG responses in mice following subcutaneous administration of 1-10 .mu.m 50:50 DL-**PLG**, 100:0 L-**PLG**, and a mixture of 50:50 DL-**PLG** and 100:0 L-**PLG** SEB toxoid-containing microcapsules.

DETD . . . delivery of the antigens (trinitrophenyl keyhole limpet hemocyanin and a toxoid vaccine of staphylococcal enterotoxin B), and a drug (etretinate) **encapsulated** in 50:50 poly(DL-**lactide**-co-**glycolide**) to mice.

DETD It should be noted, however, that other polymers besides poly(DL-**lactide**-co-**glycolide**) may be used. Examples of such polymers include, but are not limited to, poly(**glycolide**), poly(DL-**lactide**-co-**glycolide**), copolyoxalates, polycaprolactone, poly(**lactide**-co-caprolactone), poly(esteramides), polyorthoesters and poly(B-hydroxybutyric acid), and polyanhydrides.

DETD B. Preparation of **Antigen**-Loaded Microcapsules

DETD TNP-KLH, a water-soluble **antigen**, was **encapsulated** in poly(DL-**lactide**-co-**glycolide**), a biocompatible, biodegradable polyester. The procedure used to prepare the microcapsules follows:

DETD First, a polymer solution was prepared by dissolving 0.5 g. of 50:50 poly(DL-**lactide**-co-**glycolide**) in 4.0 g. of methylene chloride. Next, 300 microliters of an aqueous solution of TNP-KLH (46 mg TNP-LKH/mL; after dialysis) was added to and homogeneously dispersed in the poly(DL-**lactide**-co-**glycolide**) solution by vortexing the mixture with a Vortex-Genie 2 (Scientific Industries, Inc., Bohemia, N.Y.).

DETD The TNP-KLH content of the **antigen**-loaded microcapsules, that is, the core loading of the microcapsules, was determined by weighing out 10 mg of **antigen**-loaded microcapsules in a 12-mL centrifuge tube. Add 3.0 mL of methylene chloride to the tube and vortex to dissolve the poly(DL-**lactide**-co-**glycolide**). Next, add 3.0 mL of deionized water to the tube and vortex vigorously for 1 minute. Centrifuge the contents of. . .

DETD . . . of lymphoreticular tissue are located along the entire length of the small intestine and appendix. The targeted delivery of intact **antigen** directly into this tissue to achieve high local concentration is currently believed to be the most effective means of inducing. . .

DETD 85:15 Poly(DL-**lactide**-co-**glycolide**) Microcapsules

DETD . . . a suspension in tap water via a gastric tube. The microcapsule wall material chosen for these studies consisted of 85:15 poly(DL-**lactide**-co-**glycolide**) due to its ability to resist significant bioerosion for a period of six weeks. At various times from 1 to. . .

DETD In additional experiments, tissue sections from Peyer's patches, mesenteric lymph node and spleen which contained absorbed 85:15 DL-**PLG** microcapsules were examined by histochemical and immunohistochemical techniques. Among other observations, these studies clearly showed that the microcapsules which were. . . by periodic acid Schiff's reagent (PAS) for intracellular carbohydrate, most probably glycogen, and for major histocompatibility complex (MHC) class II **antigen**. Further, the microcapsules observed in the mesenteric lymph nodes and in the spleen were universally found to have been carried there within these PAS and MHC class II positive cells. Thus, the **antigen** containing microcapsules have been internalized by **antigen**-presenting accessory cells (APC) in the Peyer's patches, and these APC have disseminated the antigenmicrocapsules to other lymphoid tissues.

DETD . . . the size of the particles. Microcapsules <5 micrometers in diameter extravasate from the Peyer's patches within APC and release the **antigen** in lymphoid tissues which are inductive sites for systemic immune responses. In contrast, the microcapsules 5 to 10 micrometers in diameter remain in the Peyer's patches, also within APC, for extended time and release the **antigen** into this sIgA inductive site.

DETD . . . materials chosen for these studies consisted of polymers that varied in water uptake, biodegradation, and hydrophobicity. These polymers included polystyrene, poly(L-**lactide**), poly(DL-**lactide**), 50:50 poly(DL-**lactide**-co-**glycolide**), 85:15 poly(DL-**lactide**-co-**glycolide**), poly(hydroxybutyric acid), poly(methyl methacrylate), ethyl cellulose,

cellulose acetate hydrogen phthalate, and cellulose triacetate. Microcapsules, prepared from 7 of the 10. . . hours after oral administration of a suspension containing 20 mg of microcapsules, as shown in Table 3. None of the **microspheres** were seen to penetrate into tissues other than the Peyer's patches. With one exception, ethyl cellulose, the efficiency of absorption. . . group of compounds [poly(styrene), poly(methyl methacrylate), poly(hydroxybutyrate)], while 200 to 1,000 microcapsules were observed with the relatively less hydrophobic polyesters [poly(L-**lactide**), poly(DL-**lactide**), 85:15 poly(DL-**lactide**-co-**glycolide**), 50:50 poly(DL-**lactide**-co-**glycolide**)]. As a class, the cellulosics were not absorbed.

DETD . . . Michalek, S. M. and McGhee, J. R. LPS regulation of the immune response: Suppression of immune response to orally-administered T-dependent **antigen**. J. Immunol. 127:1052; 1981).

DETD Research in our laboratories has shown that microencapsulation results in a profoundly heightened immune response to the incorporated **antigen** or vaccine in numerous experimental systems. An example is provided by the direct comparison of the level and isotype distribution. . . with either soluble or microencapsulated enterotoxoid. Groups of mice were administered various doses of the toxoid vaccine incorporated in 50:50 poly(DL-**lactide**-co-**glycolide**) microcapsules, or in soluble form, by intraperitoneal (IP) injection. On Days 10 and 20 following immunization, plasma samples were obtained. . .

DETD One hundred micrograms of enterotoxoid in **microspheres** administered by SC injection at 4 sites along the backs of mice stimulated a peak IgG anti-toxin response equivalent to. . .

DETD When considering the mechanism through which 1-10 micrometer DL-**PLG microspheres** mediate a potentiated humoral immune response to the **encapsulated antigen**, three mechanisms must be considered as possibilities. First, the long term chronic release (depot), as compared to a bolus dose of nonencapsulated **antigen**, may play a role in immune enhancement. Second, our experiments have shown that **microspheres** in this size range are readily phagocytized by **antigen** processing and presenting cells. Therefore, targeted delivery of a comparatively large dose of nondegraded **antigen** directly to the cells responsible for the initiation of immune responses to T cell-dependent antigens must also be considered. Third,. . . Immunopotentiation by this latter mechanism has the characteristic that it is expressed when the adjuvant is administered concurrently with the **antigen**.

DETD In order to test whether **microspheres** possess any innate adjuvancy which is mediated through the ability of these particles to nonspecifically activate the immune system, the. . . of microencapsulated enterotoxoid was compared to that induced following the administration of an equal dose of enterotoxoid mixed with placebo **microspheres** containing no **antigen**. The various **antigen** forms were administered by IP injections into groups of 10 BALB/c mice and the plasma IgM and IgG enterotoxin-specific antibody. . .

DETD . . . IgG isotypes which was still increasing on day 30 after immunization. Co-administration of soluble enterotoxoid and a dose of placebo **microspheres** equal in weight, size and composition to those used to administer **encapsulated antigen** did not induce a plasma anti-toxin response which was significantly higher than that induced by soluble **antigen** alone. This result was not changed by the administration of the soluble **antigen** 1 day before or 1, 2 or 5 days after the placebo **microspheres**. Thus, these data indicate that the immunopotentiation expressed when **antigen** is administered within 1-10 micrometer DL-**PLG microspheres** is not a function of the ability of the **microspheres** to intrinsically activate the immune system. Rather, the data are consistent with either a depot effect, targeted

delivery of the **antigen** to **antigen**-presenting accessory cells, or a combination of these two mechanisms.

DETD Retarding the **Antigen** Release Rate from 1-10 Micrometer Microcapsules Increases the Level of the Antibody Response and Delays the the Peak Response

DETD Four enterotoxoid containing microcapsule preparations with a variety of **antigen** release rates were compared for their ability to induce a plasma anti-toxin response following IP injection. The rate of **antigen** release by the microcapsules used in this study is a function of two mechanisms; diffusion through pores in the wall. . . of the rate at which the wall materials are hydrolyzed. However, these latter two lots differ in the ratio of **lactide** to **glycolide** composing the microcapsules, and the greater resistance of the 85:15 DL-**PLG** to hydrolysis results in a slower rate of enterotoxoid release.

DETD . . . 45 which were substantially higher (102,400) than those induced by either lot with early release. Further delaying the rate of **antigen** release through the use of an 85:15 ratio of **lactide** to **glycolide**, Batch #928-060-00 (0% release at 48 hours) delayed the peak antibody levels until days 45 and 60, but no further. . .

DETD These results are consistent with a delayed and sustained release of **antigen** stimulating a higher antibody response. However, certain aspects of the pattern of responses induced by these various **microspheres** indicate that a depot effect is not the only mechanism of immunopotentiation. The faster the initial release, the lower the peak antibody titer. These results are consistent with a model in which the **antigen** released within the first 48 hours via diffusion through pores is no more effective than the administration of soluble **antigen**. Significant delay in the onset of release to allow time for phagocytosis of the **microspheres** by macrophages allows for the effective processing and presentation of the **antigen**, and the height of the resulting response is governed by the amount of **antigen** delivered into the presenting cells. However, delay of **antigen** release beyond the point where all the **antigen** is delivered into the presenting cells does not result in further potentiation of the response, it only delays the peak. . .

DETD It has been consistently observed that the size of the **microspheres** has a profound effect on the degree to which the antibody response is potentiated and the time at which it is initiated. These effects are best illustrated under conditions of a limiting **antigen** dose. Mice immunized subcutaneously with 10 .mu.g of SEB toxoid **encapsulated** in 1-10 .mu.m **microspheres** produced a more rapid, and a substantially more vigorous, IgG anti-toxin response than did mice immunized with the same dose of toxoid in 10-110 .mu.m **microspheres** as shown in FIG. 1. Groups of 5 mice were subcutaneously immunized with 10 .mu.g of SEB toxoid **encapsulated** in 1-10 .mu.m (85:15 DL-**PLG**; 0.065 wt % SEB toxoid) or 10-110 .mu.m (85:15 DL-**PLG**; 1.03 wt % SEB toxoid) **microspheres**. Plasma samples were obtained at 10 day intervals and the IgG anti-toxin titer determined by end-point titration in a RIA. . .

DETD A likely explanation for these effects involves the manner in which these different sizes of **microspheres** deliver **antigen** into the draining lymphatics. We have observed fluorescent DL-**PLG microspheres** of <10 .mu.m in diameter to be efficiently phagocytized and transported by macrophages into the draining lymph nodes. In contrast, larger **microspheres** (>10 .mu.m)remain localized at the site of injection. Taken together, these data suggest that the extremely strong adjuvant activity of <10 .mu.m **microspheres** is due to their efficient loading of **antigen** into accessory cells which direct the delivery of the microencapsulated **antigen** into the draining lymph nodes.

DETD . . . and a third injection is given to afford a tertiary response. Multiple injections are needed because repeated interaction of the **antigen** with immune system cells is required to stimulate a strong immunological response. After receiving the first injection of vaccine, a. . .

DETD The vaccine formulation that is injected into a patient may consist of an **antigen** in association with an adjuvant. For instance, an **antigen** can be bound to alum. During the first injection, the use of the **antigen**/adjuvant combination is important in that the adjuvant aids in the stimulation of an immune response. During the second and third injections, the administration of the **antigen** improves the immune response of the body to the **antigen**. The second and third administrations or subsequent administrations, however, do not necessarily require an adjuvant.

DETD Alza Corporation has described methods for the continuous release of an **antigen** and an immunopotentiator (adjuvant) to stimulate an immune response (U.S. Pat. No. 4,455,142). This invention differs from the Alza patent in at least two important manners. First, no immunopotentiator is required to increase the immune response, and second, the **antigen** is not continuously released from the delivery system.

DETD The present invention concerns the formulation of vaccine (**antigen**) into microcapsules (or **microspheres**) whereby the **antigen** is **encapsulated** in biodegradable polymers, such as poly(DL-**lactide**-co-**glycolide**). More specifically, different vaccine microcapsules are fabricated and then mixed together such that a single injection of the vaccine capsule mixture improves the primary immune response and then delivers **antigen** in a pulsatile fashion at later time points to afford secondary, tertiary, and subsequent responses.

DETD . . . the small microcapsules are efficiently recognized and taken up by macrophages. The microcapsules inside of the macrophages then release the **antigen** which is subsequently processed and presented on the surface of the macrophage to give the primary response. The larger microcapsules,. . . preferably less than 250 micrometers, are made with different polymers so that as they biodegrade at different rates, they release **antigen** in a pulsatile fashion.

DETD Furthermore, the mixture of microcapsules may consist entirely of microcapsules sized less than 10 micrometers. **Microspheres** less than 10 micrometers in diameter are rapidly phagocytized by macrophages after administration. By using mixtures of **microspheres** less than 10 micrometers in diameter that have been prepared with polymers that have various **lactide**/**glycolide** ratios, an immediate primary immunization as well as one or more discrete booster immunizations at the desired intervals (up to approximately eight months after administration) can be obtained. By mixing **microspheres** less than 10 micrometers in diameter (for the primary immunization) with **microspheres** greater than 10 micrometers in diameter, the time course possible for delivery of the discrete booster immunizations can be extended up to approximately 2 years. This longer time course is possible because the larger **microspheres** are not phagocytized and are therefore degraded at a slower rate than are the less than 10 micrometer **microspheres** .

DETD Using the present invention, the composition of the **antigen** microcapsules for the primary response is basically the same as the composition of the **antigen** microcapsules used for the secondary, tertiary, and subsequent responses. That is, the **antigen** is **encapsulated** with the same class of biodegradable polymers. The size and pulsatile release properties of the **antigen** microcapsules then maximizes the immune response to the **antigen**.

DETD The preferred biodegradable polymers are those whose biodegradation rates can be varied merely by altering their monomer ratio, for example,

poly(DL-**lactide**-co-**glycolide**), so that **antigen** microcapsules used for the primary response will biodegrade faster than **antigen** microcapsules used for subsequent responses, affording pulsatile release of the **antigen**.

DETD . . . by controlling the size of the microcapsules of basically the same composition, one can maximize the immune response to an **antigen**. Also important is having small microcapsules (microcapsules less than 10 micrometers, preferably less than 5 micrometers, most preferably 1 to 5 micrometers) in the mixture of **antigen** microcapsules to maximize the primary response. The use of an immune enhancing delivery system, such as small microcapsules, becomes even. . .

DETD . . . response of mice immunized with a single administration of JE vaccine consisting of one part unencapsulated vaccine and two parts **encapsulated** vaccine. The JE microcapsules were >10 micrometers. The results of immunizing mice with JE vaccine by these two methods were. . .

DETD . . . 42 (standard schedule) and (4) mice which received 3.0 mg of JE vaccine (unencapsulated) and 3.0 mg of JE vaccine (**encapsulated**) on day 0 were studied. The untreated controls provide background virus neutralization titers against which immunized animals can be compared.. . . dose of JE vaccine on Day 0 provide background neutralization titers against which animals receiving unencapsulated vaccine in conjunction with **encapsulated** vaccine can be compared. This comparison provides evidence that the administration of **encapsulated** vaccine augments the immunization potential of a single 3.0 mg dose of unencapsulated vaccine. The animals receiving 3 doses of unencapsulated vaccine provide controls against which the **encapsulated** vaccine group can be compared so as to document the ability of a single injection consisting of both nonencapsulated and **encapsulated** vaccine to produce antiviral activity comparable to a standard three dose immunization schedule.

DETD . . . mean titer for this group decreased by greater than 50% from Day 40 to Day 77. All ten animals receiving **encapsulated** JE vaccine (Group 4) developed serum antiviral activity. The geometric mean titer for this group increased from Day 21 to. . . no significant difference in the average titer for these two groups in the Day 77 samples (p=0.75) indicating that the **encapsulated** vaccine group achieved comparable serum antiviral titers at Day 77. Unlike the 3 vaccine dose group (Group 3), the animals receiving **encapsulated** vaccine (Group 4) continued to demonstrate increases in serum virus neutralizing activity throughout the timepoints examined. In contrast to the standard vaccine treatment group, mice receiving **encapsulated** JE vaccine had a two-fold increase in the average serum neutralizing titer from Day 49 to Day 77. The Day. . . virus neutralizing titers similar to those produced by standard vaccine administration can be achieved by administering a single dose of **encapsulated** JE vaccine. Although the antiviral titers achieved with the excipient formulation used in this study did not increase as rapidly. . .

DETD . . . JE virus. The results of these assays, presented in Table 12, substantiate the findings described above. Although the animals receiving **encapsulated** vaccine did not reach peak titers as rapidly as did the standard vaccine group, the **encapsulated** vaccine did induce comparable virus neutralizing antibody activity. Furthermore, the **encapsulated** vaccine maintained a higher antiviral titer over a longer period of time than did the standard vaccine. These results further. . .

DETD . . . and/or rate at which the incorporated material is released. In the case of vaccines this allows for scheduling of the **antigen** release in such a manner as to maximize the antibody response following a single administration. Among the possible release profiles. . .

DETD The possibility of using size as a mechanism to control vaccine release

is based on the observation that **microspheres** <10 micrometers in diameter are phagocytized by macrophages and release **antigen** at a substantially accelerated rate relative to **microspheres** made of the same DL-**PLG** but which are too large to be phagocytized. The possibility of using size to achieve pulsed vaccine release was investigated by systemically (subcutaneously) injecting 100 micrograms of enterotoxoid to groups of mice either in 1-10 micrometer (50:50 DL-**PLG**; 1.51 wt % enterotoxoid), 20-50 micrometer (50:50 DL-**PLG**; 0.64 wt % enterotoxoid) or in a mixture of 1-10 micrometer and 20-50 micrometer microcapsules in which equal parts of. . .

DETD . . . through the co-administration of 1-10 and 20-50 micrometer enterotoxoid-containing microcapsules is consistent with a two phase (pulsed) release of the **antigen**. The first pulse results from the rapid ingestion and accelerated degradation of the 1-10 micrometer particles by tissue histiocytes, which results in a potentiated primary immune response due to the efficient loading of high concentrations of the **antigen** into these accessory cells, and most probably their activation. The second phase of **antigen** release is due to the biodegradation of the 20-50 micrometer microcapsules, which are too large to be ingested by phagocytic cells. This second pulse of **antigen** is released into a primed host and stimulates an anamnestic immune response. Thus, using the 50:50 DL-**PLG** copolymer, a single injection vaccine delivery system can be constructed which potentiates antibody responses (1-10 micrometer microcapsules), and which can. . .

DETD The hydrolysis rate of the DL-**PLG** copolymer can be changed by altering the **lactide**-to-**glycolide** ratio. This approach to the pulsed release of vaccine antigens was investigated in experiments in which groups of mice were subcutaneously immunized with 10 .mu.g of SEB toxoid in 1 to 10 micrometer **microspheres** formulated from DL-**PLG** with **lactide**-to-**glycolide** ratios of 50:50 or 85:15 DL-**PLG** or 100:0 L-**PLG**. Determination of the plasma IgG anti-toxin levels in these mice as a function of time demonstrated that these preparations of. . . at distinctly different times as shown in FIG. 3. Each preparation stimulated a peak IgG titer of 409,600, but the **microspheres** formulated of 50:50 and 85:15 DL-**PLG** and 100:0 L-**PLG** resulted in this level being attained on days 50, 130 and 230, respectively.

DETD The possibility of using a blend of 1 to 10 .mu.m **microspheres** with different DL-PLGs having different **lactide**/**glycolide** ratios to deliver discrete pulsed releases of **antigen** was investigated in a group of mice subcutaneously immunized in parallel. This blend consisted of 50:50 DL-**PLG** and 100:0 L-**PLG microspheres** in which each component contained 5 .mu.g of SEB toxoid. The plasma IgG anti-SEB toxin response induced by this mixture. . . FIG. 4. The first component of this response was coincident with that seen in mice which received only the 50:50 DL-**PLG microspheres**, while the second component coincided with the time at which the immune response was observed in mice receiving only the 100:0 L-**PLG microspheres**. The anamnestic character of the second phase indicates that distinct primary and secondary anti-SEB toxin responses have been induced.

DETD These data show that in a mixture of **microspheres** with differing **lactide/glycolide** ratios, the degradation rate of an individual **microsphere** is a function of its **lactide/glycolide** ratio and that it is independent of the degradation rate of the other **microspheres** in the mixture. This finding indicates that 1) the time at which any vaccine pulse can be delivered is continuously variable across the range of **lactide/glycolide** rations, 2) the pulsed vaccine release profiles of any combination of **microspheres** with

differing **lactide/glycolide** ratios can be predicted with a high degree of certainty based on the behavior of the individual components, and 3) the delay in vaccine release possible with **microspheres** <10 .mu.m in diameter is up to approximately 8 months while the delay possible for **microspheres** >10 .mu.m is up to approximately 2 years, allowing for any number of discrete pulsatile vaccine releases over these time. . .

DETD . . . 5 micrometers, that will be engulfed by macrophages and obviate the need for immunopotentiators, as well as mixtures of free **antigen** for a primary response in combination with microcapsulated **antigen** in the form of microcapsules having a diameter of 10 micrometers or greater that release the **antigen** pulsatile to potentiate secondary and tertiary responses and provide immunization with a single administration. Also, a combination of small microcapsules. . .

DETD Orally-Administered **Microspheres** Containing TNP-KLH Induce Concurrent Circulating and Mucosal Antibody Responses to TNP

DETD Microcapsules containing the haptenated protein **antigen** trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) were prepared using 50:50 DL-**PLG** as the excipient. These microcapsules were separated according to size and those in the range of 1 to 5 micrometers in diameter were selected for evaluation. These microcapsules contained 0.2% **antigen** by weight. Their ability to serve as an effective **antigen** delivery system when ingested was tested by administering 0.5 mL of a 10 mg/mL suspension (10 micrograms **antigen**) in bicarbonate-buffered sterile tap water via gastric incubation on 4 consecutive days. For comparative purposes an additional group of mice. . .

DETD . . . administration of 30 micrograms of microencapsulated TNP-KLH in equal doses over 3 consecutive days resulted in the appearance of significant **antigen**-specific IgA antibodies in the secretions, and of all isotypes in the serum by Day 14 after immunization (see the last. . . These antibody levels were increased further on Day 28. In contrast, the oral administration of the same amount of unencapsulated **antigen** was ineffective at inducing specific antibodies of any isotype in any of the fluids tested.

DETD These results are noteworthy in several respects. First, significant **antigen**-specific IgA antibodies are induced in the serum and mucosal secretions, a response which is poor or absent following the commonly. . . mucosa; the portal of entry or site of pathology for a number of bacterial and viral pathogens. Secondly, the microencapsulated **antigen** preparation was an effective immunogen when orally administered, while the same amount of unencapsulated **antigen** was not. Thus, the microencapsulation resulted in a dramatic increase in efficacy, due to targeting of and increased uptake by. . . the absence of adjuvants is characterized by a peak in antibody levels in 7 to 14 days, the orally administered **antigen**-containing microcapsules induced responses were higher at Day 28 than Day 14. This indicates that bioerosion of the wall materials and release of the **antigen** is taking place over an extended period of time, and thus inducing a response of greater duration.

DETD . . . of mice were immunized with 100 micrograms of Staphylococcal enterotoxoid B in soluble form or within microcapsules with a 50:50 DL-**PLG** excipient. These mice were administered the soluble or microencapsulated toxoid via gastric tube on three occasions separated by 30 days,. . .

DETD These data demonstrate that microencapsulation allowed an immune response to take place against the **antigen** SEB toxoid following administration into the respiratory tract while the nonencapsulated **antigen** was ineffective. This response was observed both in the circulation and in the secretions bathing the respiratory tract. It should. . .

DETD In both man and animals, it has been shown that systemic immunization coupled with mucosal presentation of **antigen** is more effective

than any other combination in promoting mucosal immune responses (Pierce, N. F. and Gowans, J. L. Cellular. . . either the IP, oral or IT routes. This was done to directly determine if a mixed immunization protocol utilizing microencapsulated **antigen** was advantageous with respect to the levels of sIgA induced.

DETD . . . antibody responses. Although the experiments reported here examine discrete priming and boosting steps which each required an administration of microencapsulated **antigen**, it will be possible to use the flexibility in controlled pulsatile release afforded by the microcapsule delivery system to design a single time of administration regimen which will stimulate maximum concurrent systemic and secretory immunity. As an example, microencapsulated **antigen** could be administered by both injection and ingestion during a single visit to a physician. By varying the **lactide** to **glycolide** ratio in the two doses, the systemically administered dose could be released within a few days to prime the immune. . .

DETD . . . absorption of pharmaceuticals as well as antigens into the body. Etretinate, (All-E)-9-(4-methoxy-2,3,6,-trimethyl) phenyl-3,7-dimethyl-2,4,8-nonatetraenoic acid, ethyl ester) was microencapsulated in 50:50 poly(DL-**lactide**-co-**glycolide**). The microcapsules were 0.5 to 4 micrometers in diameter and contained 37.2 wt % etretinate. These etretinate microcapsules, as well. . .

DETD TABLE 1

Penetration of Coumarin-6 85:15 DL-PTG **Microspheres** Into and Through the Peyer's Patches Following Oral Administration

Time (days)	Total number	Proportion of diameter (%) Small	Medium	Large	Proportion at location (%)
. . .					

DETD TABLE 2

Migration of Coumarin-6 85:15 DL-**PLG Microspheres** Into and Through the Mesenteric Lymph Nodes Following Oral Administration

Time	Total number	Proportion of diameter (%) Small	Medium	Large	Proportion at location. . .

DETD TABLE 3

Targeted Absorption of 1- to 10-um **Microspheres** with Various Excipients by the Peyer's Patches of the Gut-Associated Lymphoid Tissues Following Oral Administration

Microsphere Excipient	Biodegradable	Absorption by the Peyer's patches
Poly(styrene)	No	Very Good
Poly(methyl methacrylate)	No	Very Good
Poly(hydroxybutyrate)	Yes	Very Good
Poly(DL-**lactide**)	Yes	Good
Poly(L-**lactide**)	Yes	Good
85:15 Poly(DL-**lactide**-co-**glycolide**)	Yes	Good
50:50 Poly(DL-**lactide**-co-**glycolide**)	Yes	Good
Cellulose acetate hydrogen phthalate	No	None
Cellulose triacetate	No	None

Ethyl cellulose No None

DETD TABLE 8

Secondary Systemic Anti-Toxin Response Induced by Various Parenteral Immunization Routes

Dose (.mu.g) Micro-**encapsulated** Toxoid per Immunization	Immunization Route	Plasma IgG Anti-Toxin Titer Day 15	Day 30	Day 45
100	IP - IP	819,200	1,638,400	3,276,800
100	SC -. . .			

DETD TABLE 9

Microspheres Do not Possess Inherent Adjuvant Activity

Dose (.mu.g) of Toxoid	Form	Plasma Anti-Toxin Titer Day 10 IgM	Day 10 IgG	Day 20 IgM	Day 20 IgG	Day 30 IgM	Day 30 IgG
25	**Antigen** in	6,400	6,400	400	12,800	800	25,600
	Microspheres						
25	Soluble **Antigen**	800	<50	200	800	100	<50
25	**Antigen** plus Placebo **Microspheres**	800	<50	200	<50	200	50

DETD TABLE 10

Systemic Anti-Toxin Response Induced by Parenteral Immunization .mu.m **Microspheres** Releasing **Antigen** at Various Rates

Dose (.mu.g) of Toxoid	Form	**Lactide/Glycolide** Ratio	**Antigen** release at 48 Hr	Plasma IgG Anti-Toxin Titer on Day 10	15	20	30	45	60
100	Soluble								
.	.	.							

DETD TABLE 14

Plasma IgM and IgG Anti-Toxin Levels on Day 20 Following Primary, Secondary, and Tertiary Oral Immunization with Soluble or Microencapsulated (50:50 DL-**PLG**) Staphylococcal Toxoid

Enterotoxoid does (.mu.g) per immunization	Form	Plasma anti-toxin titer on day 20 following oral immunization Primary IgM	Primary IgG	Secondary IgM	Secondary IgG	Tertiary IgM.	Tertiary .	Tertiary .

CLM What is claimed is:

. . . response, comprising parenterally administering an immunogenically effective amount of microcapsules to said animal, wherein said microcapsules comprise said bioactive agent **encapsulated** in a biocompatible excipient and wherein said microcapsules are of a size of between approximately 1 micrometer and approximately 10. . .
3. The method of claim 1, wherein said bioactive agent is an immunomodulator, lymphokine, monokine, cytokine, or **antigen**.

4. The method of claim 1, wherein said bioactive agent is an **antigen**.

5. The method of claim 4, wherein said **antigen** is an allergen, viral **antigen**, bacterial **antigen**, protozoan **antigen**, or a fungal **antigen**.

6. The method of claim 4, wherein said **antigen** is an influenzae **antigen**, Staphylococcus **antigen**, respiratory syncytial **antigen**, parainfluenza virus **antigen**, Hemophilus influenza **antigen**, Bordetella pertussis **antigen**, Neisseria gonorrhoea **antigen**, Streptococcus pneumoniae **antigen**, Plasmodium falciparum **antigen**, helminthic pathogen **antigen**, or an **antigen** to vaccinate against allergies.

7. The method of claim 4, wherein said **antigen** is an influenza virus or staphylococcal enterotoxin B.

15. The method of claim 1, wherein said biocompatible excipient is a poly(**lactide**-co-**glycolide**), poly(**lactide**), poly(**glycolide**), copolyoxalate, polycaprolactone, poly(**lactide**-co-caprolactone), poly(esteramide), polyorthoester, poly(.beta.-hydroxybutyric acid), polyanhydride, or a mixture thereof.

. . . response, comprising parenterally administering an immunogenically effective amount of microcapsules to said animal, wherein said microcapsules comprise said bioactive agent **encapsulated** in a biocompatible excipient and wherein said microcapsules are of a size of less than approximately 10 micrometers.

AN 1998:118870 USPATFULL|
TI Method for delivering bioactive agents into and through the mucosally associated lymphoid tissues and controlling their release|
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5814344 19980929
AI US 4692187 19950606 (8)
RLI Continuation of Ser. No. 116484, filed on 7 Sep 1993 which is a continuation of Ser. No. 629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. 325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. 169973, filed on 18 Mar 1988, now patented, Pat. No. 5075109 which is a continuation-in-part of Ser. No. 923159, filed on 24 Oct 1986, now abandoned
DT Utility|
FS Granted|
EXNAM Primary Examiner: Lovering, Richard D.|
LREP Needle & Rosenberg, P.C.|
CLMN Number of Claims: 16|

ECL Exemplary Claim: 1|
DRWN 4 Drawing Figure(s); 2 Drawing Page(s)|
LN.CNT 2121|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 34 OF 40 USPATFULL

SUMM . . . ethylene vinyl acetate fibers loaded with tetracycline described in the European patent application No. 84401985.1 to Goodson, and the biodegradable **microspheres** and matrix described in U.S. Pat. No. 4,685,883 to Jernberg. All of these delivery systems involve placing the product directly. . .

SUMM The polymeric delivery system may consist of **microspheres**, microcapsules, nanoparticles, liposomes, fibers, rods, films, or spheres. They may be fabricated from either biodegradable or nonbiodegradable polymers although delivery. . . not require removal after the chemotherapeutic agent has been released. Also preferred are the delivery systems in the form of **microspheres**, microcapsules, nanoparticles, and liposomes which can be injected directly into the gingival tissue. Liquid polymeric systems that can be injected. . .

DETD . . . periodontal disease by the use of an intragingival polymeric controlled delivery system. The polymeric delivery system in the form of **microspheres**, microcapsules, nanoparticles, or liposomes are injected directly into the infected gingival tissue where they release an active agent such as. . .

DETD . . . microencapsulation. Although microencapsulation can be used to coat drug/polymer particles already formed, it can also be used directly to form **microspheres** or microcapsules containing drug using a variety of methods known to those skilled in the art. These include solvent evaporation,. . . polymer used for the coating, the uniformity of the coating, the thickness of the coating, and the size of the **microspheres** or microcapsules can be used to control the release of drug.

DETD Other small particles which can be used for injection include liposomes. These drug delivery forms are formed by **encapsulating** various drugs in lipid bilayers. The liposomes formed are extremely small and can be injected easily into the body or. . .

DETD Poly(DL-**lactide**) (DL-PLA) with an inherent viscosity of 0.26 dL/g and a theoretical molecular weight of approximately 10,000 daltons was prepared by the ring-opening polymerization of DL-**lactide** using lauryl alcohol as the initiator and stannous chloride as the catalyst. The polymer was dissolved in N-methyl-2-pyrrolidone to give. . .

DETD Poly(DL-**lactide**-co-**glycolide**) was prepared by the ring-opening polymerization of a mixture of DL-**lactide** and **glycolide** using lauryl alcohol as the initiator and stannous chloride as the catalyst. The proportions of the two monomers were adjusted so that the final copolymer (DL-**PLG**) had a 50:50 ratio of the two monomers as determined by nuclear magnetic resonance spectrophotometry. The initiator was also adjusted. . .

DETD Tetracycline hydrochloride was added to the same DL-**PLG** solution as described in Example 5 to give a 2% by weight dispersion. After standing overnight, the drug dissolved completely. . .

CLM What is claimed is:

. . . an antimicrobial agent, an antibiotic agent, an anti-inflammatory agent, an anti-infective agent, a peptide, a protein, a growth factor, an **antigen**, and a biological response modifier.

4. A method according to claim 3 wherein the particulate form is **microspheres**, microcapsules, nanoparticles, or liposomes.

. . . acetate, chlorhexidine diacetate, chlorhexidine gluconate, tetracycline, and tetracycline hydrochloride; and wherein said polymer is selected from the group consisting of poly(DL-**lactide**) and

poly(DL-**lactide**-co-**glycolide**).

AN 94:55340 USPATFULL|
TI Intragingival delivery systems for treatment of periodontal disease|
IN Dunn, Richard L., Fort Collins, CO, United States
Tipton, Arthur J., Fort Collins, CO, United States
Harkrader, Ronald J., Louisville, CO, United States
Rogers, Jack A., Fort Collins, CO, United States
PA Vipont Pharmaceutical, Inc., New York, NY, United States (U.S. corporation)
PI US 5324520 19940628
AI US 1993-46396 19930413 (8)
RLI Continuation of Ser. No. US 1991-742719, filed on 5 Aug 1991, now abandoned which is a continuation of Ser. No. US 1988-286456, filed on 19 Dec 1988, now abandoned
DT Utility|
FS Granted|
EXNAM Primary Examiner: Michl, Paul R.; Assistant Examiner: Azpuru, Carlos|
LREP Merchant, Gould, Smith, Edell, Welter & Schmidt|
CLMN Number of Claims: 15|
ECL Exemplary Claim: 1|
DRWN No Drawings
LN.CNT 462|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 35 OF 40 USPATFULL
SUMM . . . athymic murine spleen cell cultures (J. Watson et al, Immunological Rev (1980) 51:257-258). Specifically, in the presence of IL-2 and **antigen**, certain T.sub.h cells are stimulated which then are able to contribute to antibody responses. Presumably this occurs because IL-2 is involved in the **antigen**-dependent maturation of T.sub.h cells in these nude mouse spleen cultures.
SUMM . . . that IL-2 behaves in some manner in vivo to mediate a successful immune response, including a response to a specific **antigen**, and in vitro studies have shown that cross-species reactivity of hIL-2 is very diverse (prior in vivo cross-species studies have. . .
SUMM . . . agent. Further, even when the device is not especially inflammatory, a foreign-body reaction often ensues which results in the device's **encapsulation** in fibrous tissue. Such **encapsulation** impedes the drug administration, and degrades the quality of meat at the injection site.
SUMM One form of sustained-release delivery system is the microcapsule or **microsphere**. Microcapsules/spheres are essentially small particles of active compound embedded in a suitable polymer to form spheres ranging in diameter from. . . its rate of degradation (if any), its biocompatibility, the morphology of the resulting microcapsule as it degrades, etc. Microcapsule formulations **encapsulating** steroids and other agents are reported in the literature, for example, T.R. Tice et al, Pharm Tech (1984) 8:26-35; D.R.. . .
SUMM . . . such stress-related symptoms. Such formulations comprise PEGyl-IL-2 combined with a release-modulating amount of HSA and microencapsulated in a biocompatible, bioerodible poly(**lactide** -co-**glycolide**) excipient. We have found that the controlled-release formulations of the current invention, by delivering a relatively constant, effective amount of. . .
DETD . . . serum albumin. HSA is preferably used in the practice of the instant invention to stabilize and modulate PEGyl-IL-2 release from **PLG** microcapsules, although it should be understood that serum albumins from other mammalian species (e.g., bovine serum albumin--BSA) are considered equivalents. . . is a staple of commerce. A "release modulating amount" of HSA is that amount which when mixed with PEG-IL-2 and **encapsulated** in poly(lactideco-**glycolide**) **microspheres** ensures the desirable release characteristics of

the invention. The precise quantity of HSA will vary depending upon the exact form. . .

DETD The term "**PLG**" refers to poly(**lactide**-co-**glycolide**), a biodegradable polymer known in the art. **PLG** may be prepared by ring-opening polymerization of freshly prepared dimers of d,l-lactic acid (or l-lactic acid) and glycolic acid at. . . 15 psig steam pressure. The molecular weight may be determined using gel-permeation chromatography, with polystyrene standards, or by viscometric methods. **PLG** is a random copolymer, and need not contain **lactide** and **glycolide** in equimolar amounts. The polymer's solubility and degradation characteristics may be adjusted and optimized by varying the relative ratios of **lactide** and **glycolide** in the polymer. **PLG** and its preparation and use to prepare microcapsules is described in T.R. Tice et al, Pharm Tech (1984) 8:26-35; D.R.. . .

DETD The terms "**microspheres**" and "microcapsules" are used interchangeably herein, and refer to polymer particles having IL-2 or PEGyl-IL-2 contained or dispersed within. As. . . a population having diameters ranging from about 10 to about 400 um. The process employed in the instant invention produces **microspheres** having an average diameter of about 100 um, and ranging from about 70 to about 140 um. **PLG** microcapsules are biodegradable, and thus provide a three-component release profile. The first phase ("initial burst") releases loosely bound and non-**encapsulated** compound (this may be eliminated, if desired, by washing the microcapsules prior to use). In the second phase, compound diffuses through the **PLG**, or through pores in the **PLG**, which appear and enlarge as degradation of the polymer progresses. In the third phase, compound which has been trapped within. . .

DETD . . . characteristic of release curves, wherein administration is followed by an immediate, high release of compound (e.g., >8% of the total **encapsulated** protein). The initial burst is believed to be caused by the incomplete **encapsulation** of protein in microcapsules, or the degradation of microcapsules due to storage or handling. Thus, any non-**encapsulated** protein will be immediately present in solution. The initial burst may prove beneficial in some circumstances, e.g., by establishing a. . .

DETD . . . 26 June 1985, now abandoned, incorporated herein by reference. HSA is commercially available in solution and as a lyophilized powder. **PLG** may be prepared as described in D.R. Cowsar et al, Meth Enzymol (1985) 112:101-116, incorporated herein by reference.

DETD . . . PEGyl-IL-2 and HSA are combined either in solution or as finely divided powders. The PEGyl-IL-2+HSA composition is then microencapsulated in **PLG**. A solution of **PLG** in methylene chloride is stirred, and the PEGyl-IL-2+HSA composition dispersed therein. The protein composition is added in an amount sufficient. . . by a variety of methods, however, the above-described method provides particularly preferred microcapsules, having a narrow size distribution and high **encapsulation** efficiency. Other microcapsules within the scope of this invention will contain PEGyl-IL-2+HSA, (or an HSA equivalent), will be able to. . .

DETD . . . that can be injected intramuscularly or subcutaneously with a conventional hypodermic needle, and the microcapsules contain 0.5 to 20% PEGyl-hIL-2 **encapsulated** in **PLG** with a release-modulating amount of HSA.

DETD (Preparation of **PLG** Polymer)

DETD **PLG** is prepared following the procedure set forth by D.R. Cowsar et al, Meth Enzymol (1985) 112:101-116, incorporated herein by reference.. . .

DETD This polymer is prepared by ring-opening polymerization of **lactide** and **glycolide** (cyclic lactone dimers of lactic acid and glycolic acid, respectively) to form a random copolymer. **Glycolide** and **lactide** may be obtained from commercial sources, or may be prepared by dimerizing glycolic or lactic acid,

respectively, followed by pyrolysis to provide the closed-ring product. This process is illustrated below with **glycolide**.

DETD Preparation of **Glycolide**

DETD . . . reduced to 2 mmHg with a vacuum pump. The reaction flask is heated to 260.degree.-280.degree. C. to distill the crude **glycolide**. The material distilling between 110 and 130.degree. C. is collected in the first receiving flask, to provide crude **glycolide** (about 195 g).

DETD The crude **glycolide** is purified by pulverizing the mass and slurrying it with isopropanol (400 ml) at room temperature. The **glycolide** is collected by vacuum filtration, and thereafter protected from atmospheric moisture. The **glycolide** is combined with a volume of dry ethyl acetate (EtOAc, stored over molecular sieves) equal to 75% of its weight,. . . in a desiccator, to yield about 120 g of pure (>99.5% van't Hoff purity by differential scanning calorimetry - DSC) **glycolide**, m.p. 82.degree.-84.degree. C.

DETD Preparation of DL-**Lactide**

DETD Crude DL-**lactide** may be purchased from commercial suppliers, and is purified as follows:

DETD Crude DL-**lactide** (200 g) is combined with EtOAc (200 ml) in a beaker, and the mixture gently heated on a stirring hot plate to dissolve the **lactide**. The hot mixture is then quickly filtered through an extra-coarse sintered glass frit to remove insoluble material. The filtered solution is then distilled under vacuum to reduce the solvent volume to about half the weight of the **lactide**. The filtered material is then allowed to cool slowly to room temperature, and then cool for an additional 2 hours. . . is collected by vacuum filtration and dried at room temperature in a desiccator under vacuum (about 2 mmHg). The purified **lactide** is characterized and stored in an oven-dried glass jar in a desiccator until needed. The final yield is about 125. . .

DETD . . . at 150.degree. C. and cooled under dry N.sub.2. All manipulations are conducted in a glove box under dry N.sub.2. Pure **glycolide** (9.9 g) and DL-lact.d:e (90.1 g) are added to the flask (mol %=12%/88%) and heated at 140.degree.-145.degree. C. using an. . .

DETD . . . to 0.8 dl/g (0.5 g/dl in CHCl.sub.3 at 30.degree. C.). The ratio of monomers in the random copolymer is 85-86% **lactide**, 14-15% **glycolide**, as may be determined by NMR in 50:50 hexafluoroacetone:trifluoroacetic acid.

DETD (Treatment With Non-**encapsulated** IL-2)

DETD **PLG** microcapsules containing PEGyl-IL-2+HSA were prepared as follows:

DETD **PLG** (1.0003 g), was prepared as in Preparation 1, but with a **lactide**:**glycolide** ratio of 52:48 (inherent viscosity 0.73 dL/g, in hexafluoroisopropanol at 30.degree. C. using a Cannon viscometer). Then, **PLG** (0.5006 g) was weighed into a glass sample vial (6 mL), followed by CH.sub.2 Cl.sub.2 (3 7 mL). The vial. . . a 12% solution. Next, a 1:20 mixture of IL-2+HSA (0.1256 g) was weighed into a 16.times.75 mm test tube. The **PLG** solution was added to the test tube, and the mixture homogenized three times for 30 sec, with 15 sec intervals. . .

DETD . . . 5, 6, and 8 were dropped from the remainder of the comparison. Each of the remaining formulations was loaded into **PLG** microcapsules as described in Example 4. Samples of each microcapsule formulation were assayed for protein content by the method of. . .

CLM What is claimed is:

. . . for continuously delivering a relatively constant, effective amount of PEGyl-IL-2 comprising: PEGyl-IL-2 with a release-modulating amount of human serum albumin, **encapsulated** in poly(**lactide** -co-**glycolide**) microcapsules.

. . . continuously over a period of 14-30 days, which formulation comprises: PEGyl-IL-2 mixed with a release-modulating amount of human

serum albumin, **encapsulated** in poly(**lactide**-co-**glycolide**) microcapsules; and a liquid, pharmaceutically acceptable excipient capable of suspending said microcapsules.

AN 92:27517 USPATFULL|
TI Controlled-release formulations of interleukin-2|
IN Singh, Maninder, Mountain Brook, Rodeo, CA, United States
Nunberg, Jack H., Mountain Brook, Oakland, CA, United States
Tice, Thomas R., Mountain Brook, Birmingham, AL, United States
Hudson, Michael E., Mountain Brook, Gardendale, AL, United States
Gilley, Richard M., Mountain Brook, AL, CA, United States
Taforo, Terrance A., San Leandro, CA, United States
PA Cetus Corporation, Emeryville, CA, United States (U.S. corporation)
PI US 5102872 19920407
AI US 1988-231757 19880812 (7)
RLI Continuation-in-part of Ser. No. US 1986-856680, filed on 25 Apr 1986, now patented, Pat. No. US 4818769 which is a continuation-in-part of Ser. No. US 1985-778371, filed on 20 Sep 1985, now abandoned
DT Utility|
FS Granted|
EXNAM Primary Examiner: Griffin, Ronald W.|
LREP McGarrigle, Philip L., Gruber, Lewis S., Green, Grant|
CLMN Number of Claims: 6|
ECL Exemplary Claim: 1|
DRWN 24 Drawing Figure(s); 23 Drawing Page(s)|
LN.CNT 883|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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FULL ESTIMATED COST                                      90.01      90.31

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=> s l6 and heptane
L7 4 L6 AND HEPTANE

=> d l7 1-4

L7 ANSWER 1 OF 4 USPATFULL
AN 1998:64760 USPATFULL
TI Vaccines against intracellular pathogens using antigens **encapsulated** within biodegradble-biocompatible **microspheres**
IN Burnett, Paul R., Silver Spring, MD, United States
Van Hamont, John E., Ft. Meade, MD, United States
Reid, Robert H., Kensington, MD, United States
Setterstrom, Jean A., Alpharetta, GA, United States
Van Cott, Thomas C., Brookeville, MD, United States
Birx, Debrah L., Potomac, MD, United States
PA The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
PI US 5762965 19980609
AI US 1996-598874 19960209 (8)
RLI Continuation-in-part of Ser. No. US 1994-242960, filed on 16 May 1994 And Ser. No. US 1995-446149, filed on 22 May 1995 which is a continuation of Ser. No. US 1984-590308, filed on 16 Mar 1984, now abandoned , said Ser. No. US -242960 which is a continuation-in-part of Ser. No. US 1992-867301, filed on 10 Apr 1992, now patented, Pat. No. US 5417986 which is a continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990, now abandoned
DT Utility
FS Granted
LN.CNT 315
INCL INCLM: 424/499.000
INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/422.000

NCL NCLM: 424/499.000
NCLS: 424/422.000; 424/426.000; 424/455.000; 424/486.000; 424/488.000
IC [6]
ICM: A61K009-00
ICS: A61K009-66; A61K009-14; A61F013-00
EXF 424/499; 424/426; 424/455; 424/486; 424/488; 424/422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 2 OF 4 USPATFULL
AN 97:112180 USPATFULL
TI Microparticle carriers of maximal uptake capacity by both M cells and non-M cells
IN Reid, Robert H., Kensington, MD, United States
van Hamont, John E., Fort Meade, MD, United States
Brown, William R., Denver, CO, United States
Boedeker, Egar C., Chevy Chase, MD, United States
Thies, Curt, Ballwin, MO, United States
PA The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
PI US 5693343 19971202
AI US 1994-242960 19940516 (8)
RLI Continuation-in-part of Ser. No. US 1992-867301, filed on 10 Apr 1992, now patented, Pat. No. US 5417986 which is a continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990, now abandoned which is a continuation-in-part of Ser. No. US 1990-493597, filed on 15 Mar 1990, now abandoned which is a continuation-in-part of Ser. No. US 1984-590308, filed on 16 Mar 1984
DT Utility
FS Granted
LN.CNT 624
INCL INCLM: 424/491.000
INCLS: 424/493.000; 424/486.000; 424/497.000; 424/499.000; 424/501.000; 514/788.100; 514/965.000
NCL NCLM: 424/491.000
NCLS: 424/486.000; 424/493.000; 424/497.000; 424/499.000; 424/501.000; 514/788.100; 514/965.000
IC [6]
ICM: A61K009-16
ICS: A61K009-50; A61K047-30
EXF 424/491; 424/493; 424/486; 424/497; 424/499; 424/501; 424/DIG.7; 514/965
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 3 OF 4 USPATFULL
AN 97:14439 USPATFULL
TI Preparation of microparticles and method of immunization
IN O'Hagan, Derek T., 16 Middlesex Rd., Bootle, Merseyside L20 9BW, United Kingdom
McGee, John P., Tanjong Kilmarnack Rd., Kilmaurs, Strathelyde KA3 2RB, Scotland
Davis, Stanley S., 19 Cavendish Crescent North, Nottingham NG7 1BA, United Kingdom
PI US 5603960 19970218
WO 9427718 19941208
AI US 1995-374751 19950602 (8)
WO 1994-US5834 19940524
19950602 PCT 371 date
19950602 PCT 102(e) date
PRAI GB 1993-10781 19930525
DT Utility
FS Granted
LN.CNT 789

INCL INCLM: 424/501.000
INCLS: 424/451.000; 424/489.000; 264/004.100; 428/402.210; 428/402.240; 514/885.000; 514/963.000; 530/806.000
NCL NCLM: 424/501.000
NCLS: 264/004.100; 424/451.000; 424/489.000; 428/402.210; 428/402.240; 514/885.000; 514/963.000; 530/806.000
IC [6]
ICM: A61K009-50
ICS: A61K009-48; A61K009-14; B01J013-02
EXF 424/451; 424/489; 424/501; 264/4.1; 428/402.21; 428/402.24; 514/885; 514/963; 530/806
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 4 OF 4 USPATFULL
AN 95:45359 USPATFULL
TI Vaccines against diseases caused by enteropathogenic organisms using antigens **encapsulated** within biodegradable-biocompatible **microspheres**
IN Reid, Robert H., Kensington, MD, United States
Boedeker, Edgar C., Chevy Chase, MD, United States
van Hamont, John E., Shape, Belgium
Setterstrom, Jean A., Takoma Park, MD, United States
PA The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
PI US 5417986 19950523
AI US 1992-867301 19920410 (7)
RLI Continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990, now abandoned which is a continuation-in-part of Ser. No. US 1990-493597, filed on 15 Mar 1990, now abandoned which is a continuation-in-part of Ser. No. US 1984-590308, filed on 16 Mar 1984
DT Utility
FS Granted
LN.CNT 2736
INCL INCLM: 424/499.000
INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/489.000; 424/444.000; 424/433.000; 424/470.000; 424/491.000; 424/422.000
NCL NCLM: 424/499.000
NCLS: 424/422.000; 424/426.000; 424/433.000; 424/444.000; 424/455.000; 424/470.000; 424/486.000; 424/488.000; 424/489.000; 424/491.000
IC [6]
ICM: A61K009-50
ICS: A61K009-66; A61K009-26
EXF 424/499; 424/422; 424/85; 424/417; 424/450; 424/458; 424/469; 424/88; 424/89; 424/92; 424/863; 424/965
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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=> d l7 1-4 kwic

L7 ANSWER 1 OF 4 USPATFULL
TI Vaccines against intracellular pathogens using antigens **encapsulated** within biodegradble-biocompatible **microspheres**
AB This invention relates to parenteral and mucosal vaccines against diseasesaused by intracellular pathogens using antigens **encapsulated** within a biodegradable-biocompatible **microspheres**(matrix).
SUMM This invention relates to parenteral and mucosal vaccines against diseases caused by intracellular pathogens using antigens

encapsulated within biodegradable-biocompatible **microspheres**(matrix).

SUMM The issues of durability and mucosal immunogenicity have been previously addressed by **encapsulating** vaccine antigens in appropriately-sized biodegradable, biocompatible **microspheres** made of **lactide/glycolide** copolymer (the same materials used in resorbable sutures). It has been shown that such **microspheres** can be made to release their load in a controlled manner over a prolonged period of time and can facilitate. . .

SUMM . . . on the surface of both free virus and infected cells, and present it to the immune system (systemic and mucosal) **encapsulated** in **microspheres** to protect and augment its immunogenicity.

DETD This invention relates to a novel pharmaceutical composition, a microcapsule/sphere formulation, which comprises an **antigen** **encapsulated** within a biodegradable polymeric matrix, such as poly(DL-**lactide** co **glycolide**) (**PLG**), wherein the relative ratio between the **lactide** and **glycolide** component of the **PLG** is within the range of 52:48 to 0:100, and its use, as a vaccine, in the effective induction of antiviral. . . antigens. In the practice of this invention, applicants found that when a complex (oligomeric) native envelope protein of HIV-1 was **encapsulated** in **PLG** **microspheres**, it retained its native antigenicity and function upon its release in vitro. Furthermore, when used as a vaccine in animals,. . .

DETD Microencapsulation of immunogens: **PLG microspheres** ranging from 1 to 20 um in diameter and containing a 0.5 to 1.0% **antigen** core load were prepared by a solvent extractive method. The solvent extraction method involves dissolving the viral **antigen** and sucrose (1:4 ratio w:w) in 1 ml of deionized water. This solution is flash frozen and lyophilized. The resulting **antigen**-loaded sucrose particles are resuspended in acetonitrile and mixed into **PLG** copolymer dissolved in acetonitrile. This **antigen**-polymer mixture is then emulsifyed into heavy mineral oil, transferred into **heptane** and mixed for 30 min to extract the oil and acetonitrile from the nascent spheres. The spheres are harvested by centrifugation, washed three times in **heptane** and dried overnight under vacuum. **Microsphere** size was determined by both light and scanning electron microscopy. The **antigen** core load was determined by quantitative amino acid analysis of the **microspheres** following complete hydrolysis in 6N hydrochloric acid.

DETD Analysis of immunogen spontaneously released from **microspheres** in vitro by binding to soluble CD4 and recognition by HIV-positive patient serum. **PLG microspheres** loaded with native (oligomeric) gp 160 were suspended in phosphate-buffered saline, pH 7.4 (PBS), incubated at 37 C. for 3 h, and then at 4 C overnight. The **microspheres** were then sedimented by centrifugation (2 min at 200.times.g), the supernatants harvested, and the released gp 160 assayed for binding. . .

DETD Immunization of animals. HIV-seronegative, 8-10 week old NZW rabbits were immunized intramuscularly with rgp 160- or o-gp 160-loaded **PLG microspheres** suspended in PBS or with alum-adjuvanted rgp 160 in PBS. Groups receiving rgp 160-loaded **microspheres** (n=2) were primed with 50 ug of immunogen on day 0 and boosted with 25 ug on day 42. Groups receiving o-gp 160-loaded **microspheres** (n=3) were primed with 70 ug of immunogen on day 0 and boosted with 35 ug on day 56. Groups. . .

DETD BALB/c mice were immunized subcutaneously with rgp 160-loaded **PLG microspheres** suspended in PBS or with alum-adjuvanted rgp 160 in PBS. The mice in all groups (n=4) received 10 ug of. . .

DETD Comparison of the native (oligomeric) gp 160 prior to microencapsulation

and following spontaneous release from **PLG microspheres** showed the two to be essentially indistinguishable in terms of their binding to CD4 and recognition by HIV-positive patient serum. (Table 1). This retention of conformation-dependent binding shows that structure of the **antigen** is not appreciably altered by the microencapsulation process.

DETD . . . (CTL) assay performed on the speen cells of mice which had had been previously immunized with either HIV-1 envelope protein **encapsulated** in **PLG microspheres** (dark squares) or the same protein administered in a conventional way with alum adjuvant (dark diamonds). These data indicate that microencapsulation of HIV-1 envelope protein in **PLG microspheres** results in a vaccine that induces significantly greater anti-HIV CTL activity than does alum-adjuvanted vaccine. The open symbol groups represent. . .

DETD . . . binding of antibodies to native vs denatured viral protein. These data show that rabbits immunized with a non-native HIV-1 protein **encapsulated** in **PLG** (#5 and 6) develop antibodies which show greater binding to denatured (vs native) protein (indicated by a ratio<1). On the other hand, rabbits immunized with a native HIV-1 protein **encapsulated** in **PLG microspheres** (#10-12) develop antibodies which show greater binding to native viral protein (indicated by ratio>1). This retention of each proteins antigenicity constitutes an additional piece of evidence that the structure of antigens loaded in **PLG microspheres** are preserved.

DETD Microencapsulation of immunogens: **PLG microspheres** ranging from 1 to 15 um in diameter and containing a 0.5 to 1.0% **antigen** core load were prepared by a solvent evaporation method. The solvent evaporation method involves emulsifying the viral **antigen** dissolved in deionized water into poly(DL-**lactide**-co-**glycolide**) polymer dissolved in methylene chloride. This emulsion is mixed into 0.9% polyvinyl alcohol and stirred. After 10 min of stirring,. . . 1.5 h. The resulting spheres are harvested by centrifugation, washed three times in distilled water, and dried overnight under vacuum. **Microsphere** size was determined by both light and scanning electron microscopy. The **antigen** core load was determined by quantitative amino acid analysis of the **microspheres** following complete hydrolysis in 6N hydrochloric acid.

DETD Analysis of spontaneously released **antigen** showed it to retain its CD4 binding capacity. Its native antigenicity (recognition by the serum of an HIV-positive patient) was only slightly less than that of the **antigen** prior to **encapsulation** and following spontaneous release from **microspheres** produced by a solvent extraction method (Table 1).

DETD The results of immunizing animals with either non-native (denatured) or native oligomeric gp 160 in **PLG microspheres** produced by a solvent evaporation method were essentially indistinguishable from those obtained using **microspheres** produced by a solvent extraction method (example 1). Microencapsulated **antigen** induced significantly greater CTL activity than **antigen** administered in a conventional alum-adjuvanted formulation. Furthermore, preservation of the structure of **PLG** -microencapsulated antigens is supported by the findings of preferential binding of antibodies elicited by **microspheres** loaded with denatured **antigen** to denatured gp 120 (FIGS. 2, 3 and 4) and the preferred binding of antibodies elicited by **microspheres** loaded with native (oligomeric) **antigen** to native gp 120 (FIGS. 2, 7-8).

DETD TABLE 1

BIA (released o-gp160)
Capture o-gp160-451 (stock vs **microsphere**-released)

on tvc 391 fc3/fc4 sCD4 (4 mg/m)
1 ul/min flow rate for o-gp160 inj.; 5 ul/min for all others

Ilgate	RU	HIV+/sCD4 (RU ratio)
gp120-MN 1:10	3286	
HIV+ 1:100	54	
NHS 1:100	3	
HIV+ pool 1:100		
	47	
o-gp160 (tvc281)		
	1772	
HIV+	3259	1.84
tvc281	1848	
NHS	-36	
tvc281	1762	
HIV+ pool	2597	1.47
tvc281-**PLG**-EV	3342	
HIV+	4594	1.37
tvc281	3222	
NHS	7	
tvc281	3210	
HIV+ pool	3336	1.04
tvc281-**PLG**-EX	1855	
HIV+	3760	2.04
tvc281	1839	
NHS	2	
tvc281	1850	
HIV+ pool	2745	1.48
gp120-MN 1:10	2914	
HIV+ 1:100	14	
NHS 1:100	-2	
HIV+ pool 1:100		
	14	
tvc281	1099	
HIV+	1083	0.99
tvc281	1022	
HIV+ pool	1395	1.36
tvc281-**PLG**-EV	1595	
HIV+	1322	0.83
tvc281	1535	
HIV+ pool	1781	1.16

CLM What is claimed is:

1. An immunostimulating composition comprising **encapsulating microspheres** comprised of (a) a biodegradable-biocompatible poly(DL-**lactide**-co-**glycolideas** the bulk matrix produced by a solvent evaporation process wherein the molecular weight of the copolymer is between 4,000 to. . .

2. The immunostimulating composition described in claim 1 wherein the **antigen** is pre-**encapsulated** into a conformationally stabilizing hydrophilic matrix consisting of an appropriate mono, di- or tri-saccharide or other carbohydrate susbstance by lyophilization prior to its final **encapsulation** into the **PLG microsphere** by a solvent extraction process employing acetonitrile as the polymer solvent, mineral oil as the emulsion's external phase, and **heptane** as the extractant.

3. The immunostimulating compositions described in claims 1 or 2 wherein the immunogenic substance is a native (oligomeric)HIV-1 envelope **antigen** that is conformationally stabilized by the polymer matrix and serves to elicit in animals the production of HIV specific cytotoxic T lumphocytes and antibodies preferentially reactive against native HIV-1 envelope **antigen**.

5. The immunostimulating compositions describe in claim 4 wherein the relative ratio between the amount of the **lactide**: **glycolide** components of said matrix is within the range of 52:48 to 0:100.

. . . immunostimulating compositions described in claim 5, employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . in claim 5, employed as a mucosal vaccine wherein the size of more than 50% (by volume) of said vaccine **microspheres** is between 5 to 10 microns in diameter.

10. A composition in accordance with claim 1 wherein the **microspheres** further contain a pharmaceutically-acceptable adjuvant.

. . . immunostimulating compositions described in claim 6 employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . immunostimulating compositions described in claim 7 employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . in claim 6 employed as a mucosal vaccine wherein the size of more than 50% (by volume) of said vaccine **microspheres** is between 5 to 10 microns in diameter.

L7 ANSWER 2 OF 4 USPATFULL

AB In a solvent extraction process for preparing **microspheres** of a biodegrade polymer, the improvement comprising: preparing a homogenized **antigen**-sucrose matrix and adding a solvent to the sucrose-**antigen** matrix to form a solution; preparing a solution of a biodegradable polymer by adding a solvent to the polymer; adding the biodegradable polymer solution to the **antigen** -sucrose solution; adding an oil to the polymer-sucrose-**antigen** solution to form an emulsion having a controlled viscosity that corresponds to a predetermined average particle size of distributions of **microspheres** of biodegradable polymers; centrifuging the emulsion of controlled viscosity and removing the supernatant to obtain **microspheres** of a predetermined range of particle size distributions of from about 0.5 to about 7.0 micrometers.

AB An immunostimulating composition comprising an **encapsulating**-**microsphere** of the biodegradable polymer has an average particle size distribution such that the majority of the **microspheres** will be taken up by the villous epithelium section of the intestines of a mammalian subject when administered as a. . .

SUMM . . . by gut lymphoid tissues will absorb any antigens so as to induce production of antibodies against diseases caused by the **antigen** or other enteropathogenic organisms, when using antigens **encapsulated** within biodegradable-biocompatible **microspheres** prepared by the process of the invention.

SUMM . . . It is apparent from past studies that a protective mucosal immune response can best be obtained by introduction of the **antigen** at the mucosal surface; however, parenteral immunization has not been an effective method to induce mucosal immunity. **Antigen** taken up by the gut-associated lymphoid tissue (GALT), primarily by the Peyer's patches stimulates T helper cells (T.sub.H) to assist. . .

SUMM While particulate **antigen** appears to shift the responses towards the (T.sub.H), soluble antigens favor a response by the (T.sub.KS).

SUMM Although studies have demonstrated that oral immunization does induce an intestinal mucosal immune response, large doses of **antigen** are generally required to achieve sufficient local concentrations in the Peyer's patches. Further, unprotected protein antigens tend to be degraded. . .

SUMM One approach to overcoming the aforementioned problems is to homogeneously disperse the **antigen** of interest within the polymeric matrix of biodegradable, biocompatible **microspheres** that are specifically taken up by GALT. Eldridge, et al..sup.1 have used a murine model to show that orally-administered 1-10 micrometer **microspheres** consisting of polymerized **lactide** and **glycolide**, (the same materials used in resorbable sutures), were readily taken up into Peyer's patches, and that 1-5 micrometer sizes were rapidly phagocytized by macrophages. **Microspheres** that were 5-10 micrometers (microns) remained in the Peyer's patches for up to 35 days, whereas those less than 5. . .

SUMM .sup.1 Biodegradable **Microspheres**: Vaccine Delivery System For Oral Immunization, 1989, 146.

SUMM However, Eldridge, et al. used 50 .mu.m **microspheres** of poly (DL-**lactide**-co-**glycolide**) composed of molar parts of polymerized **lactide** and **glycolide** (85:15 DL-**PLG**), which biodegrades to completion in approximately 24 weeks after intramascular injection.

SUMM Poly (DL-**lactide**-co-**glycolide**) composed of equal molar parts of polymerized **lactide** and **glycolide** (50:50 DL-**PLG**) is the more stable or lest biodegradable, and biodegrades to completion after 25 weeks.

SUMM Therefore, there is a need extant in the biodegradable **microsphere** field to provide a method of producing poly (DL-**lactide**-co-**glycolide**) materials of 50:50 DL-**PLG** that is more biodegradable and capable of being taken up by both M cells and non-M cells in the Peyer's. . .

SUMM One object of the invention is to provide a method for producing microparticles of biodegradable-biocompatible **microspheres** having an average particle size distribution that maximizes uptake of the **microspheres** by both M cells and non-M cells, either in the villous epithelium or in the Peyer's patches follicle-associated epithelium so that, upon **encapsulating** antigens or other chemotherapeutic agents within these **microspheres**, large doses of **antigen** will not be required to achieve sufficient local concentrations in these regions of the intestines when these microparticles are used. . .

SUMM A further object of the invention is to provide a method for producing **microspheres** composed of poly (DL-**lactide**-co-**glycolide**) having an average particle size distribution so as to maximize the uptake of these **microspheres** into the lymphoid tissue of the gut through uptake by both M cells and non-M cells, either in the villous epithelium or in the PP follicle-associated epithelium, in order to enable smaller doses of **antigen** to achieve sufficient local concentrations in these regions of the intestines when using the poly (DL-**lactide**-co-**glycolide**) as a carrier of immunogens for oral or other types of immunization.

SUMM . . . to provide a method for producing an average distribution of particle sizes of the most stable or least biodegradable poly (DL-**lactide**-co-**glycolide**) having equal molar parts of polymerized **lactide** and **glycolide** (50:50 DL-**PLG**) so as to maximize uptake of **microspheres** of this copolymer by both M cells and non-M cells, either in the villous epithelium or in the PP follicle-associated. . .

SUMM In general the invention is accomplished by modifying the solvent extraction process for producing **microspheres** so that the average particle size distribution can be controlled by altering the viscosity of the emulsion, either by: 1). . . screen and rotor dimensions of the equipment and emulsification speed and time have

negligible effects on the outcome of the **microspheres** diameter.

SUMM FIG. 1 shows that, during preparation of the **microspheres**, the spheres actually got larger as the emulsion time was increased.

SUMM FIG. 2 is a schematic showing the preparation of sucrose-loaded vaccine placebo **microspheres**.

SUMM FIG. 5 shows that reducing the viscosity of the paraffin oil by diluting it with **heptane** resulted in the formation of progressively larger spheres.

SUMM FIG. 7 shows that when reducing the viscosity of the paraffin oil by diluting it with **heptane** using one second emulsification without an emulsion screen, resulted in the formation of progressively larger spheres.

SUMM FIG. 8 shows **microsphere** volume average versus emulsification time in paraffin oil.

SUMM FIG. 10 shows viscosity versus sphere diameter obtained with paraffin oil diluted with **heptane**.

SUMM . . . lymphoid follicle of a New Zealand white rabbit histochemically stained for acid phosphatase (red) and immunohistochemically stained for the MHCII **antigen**.

SUMM . . . the flank region of the intestinal lymphoid follicle histochemically stained for alkaline phosphasate (red) and immunohistochemically stained for the MHCII **antigen**.

SUMM . . . a color photograph of the flank region of the intestinal lymphoid follicle of a New Zealand white rabbit showing numerous **microspheres** of the poly (DL-**lactide**-co-**glycolide**) composed of molar parts of polymerized **lactide** and **glycolide** (50:50 DL-**PLG**) in the company of MHCII-positive cells in lymphoid pockets in the Follicle Associated Epithelium (FAE), and wherein some of the **microsphere** particles are within the cells (arrows). In the lymphoid follicle, numerous MHUCII-positive cells are present, and some have **microspheres** associated with them (arrowheads).

SUMM . . . kinds of particles were taken up by the follicle-associated epithelium and entered the underlying lymphoid tissues of Peyer's patches (fluoresceinated **microspheres** are more easily visualized, and as a consequence they are shown in the photograph).

SUMM FIG. 17 is an immunofluorescence micrograph of the previously illustrated lymphoid follicle. The fluorescein-labeled **microspheres** are present mostly in the flank region of the FAE (lower area of the photograph), with declining numbers present in. . .

SUMM . . . photograph showing the lymphoid follicle of a Peyer's patch of a New Zealand white rabbit stained for vimentin. The polymerized **lactide** and **glycolide** particles appear principally in the FAE area and are practically non-existent in the villous area.

DETD Use of the emulsion viscosity as the means for controlling the average particle size distribution of polymerized **lactide** and **glycolide microspheres** has utility in manufacturing oral and injectable vaccines as well as for use in devices for sustained drug and antibiotic delivery. Preparation of the **microspheres** was accomplished by a modification of the solvent extraction process to control the sphere size by altering the viscosity of. . .

DETD . . . screen and rotor dimensions, emulsification speed and time only exhibit negligible effects on the outcome of the diameter of the **microspheres**.

DETD The following examples will provide more detailed steps in producing the controlled particle size **microspheres** of poly (DL-**lactide**-co-**glycolide**) by the modified solvent extraction process of the present invention.

DETD Preparation of Freeze-Dried **Antigen**-Sucrose Matrix

DETD 20 mg purified **antigen**/active

DETD Preparation of the **Antigen**-Sucrose Matrix

DETD The **antigen**/active is placed in a 20 ml capacity plastic vial to which water and sucrose are added.

DETD Preparation of Polymerized **Lactide Glycolide** (**PLG**) Solution

DETD The **PLG** is removed from the freezer and allowed to come to room temperature.

DETD 1.5 g of acetonitrile is weighed into another vial and added to the earlier prepared freeze-dried sucrose-**antigen** matrix and mixed until it becomes a milky white slurry. The slurry is homogenized at maximum speed for one minute. . .

DETD The polymer solution prepared earlier is added to one of the vials of homogenized sucrose-**antigen** and the vial is placed in a sonicator bath for about 2 minutes to ensure proper mixing.

DETD Preparation of **Microspheres**

DETD . . . materials are weighed out: 400 g of light mineral oil in a 600 ml glass beaker and 2500 g of **heptane** in a 4000 ml propylene beaker. A beaker of **heptane** is placed under the mixer and a propeller is placed about two-thirds of the way down into the **heptane**, after which the mixer is started at about 450 rpm.

DETD . . . pump speed is set at 300 ml/min after which one end of the tubing is placed into the beaker of **heptane**.

DETD The polymer/sucrose-**antigen** solution is poured into the beaker and the vial is rinsed with about 5 ml of mineral oil, and the. . .

DETD . . . head, the homogenizer is turned off and pumping is continued until all of the liquid has been pumped to the **heptane** after which the **heptane** is left stirring for 30 minutes.

DETD Using fresh tubing, the **heptane** is pumped into centrifuge bottles and centrifuged for 5 minutes at 3000 rpm, 20 degrees celsius. The supernatant is pumped into waste bottles and the sediment is rinsed with **heptane** (it may be necessary to sonicate the sample for 1 to 5 minutes to break up the sediment).

DETD The supernatant is pumped into the waste bottle and washed with fresh **heptane** until all the **microspheres** are in the one tared 50 ml centrifuge tube. This tube is then centrifuged for 5 minutes and washed with fresh **heptane** three times.

DETD After the final wash and centrifuge cycle, the supernatant is pumped into the waste bottle and the **microspheres** are air dried with a slow air current for about 5 minutes, and the tube is placed in the vacuum. . .

DETD The **microspheres** are removed from the vacuum oven and weighed, after which about 1 mg of the **microspheres** is put in a 1.5 ml centrifuge tube for evaluation.

DETD Evaluation of **Microspheres**

DETD About 1 ml of 1% Tween 80 in water is added to the 1 mg of **microspheres** in the 1.5 ml centrifuge tube, and the tube is sonicated for about 1 minute.

DETD . . . under 100.times. magnification using a standard oil immersion technique. Using the precalibrated eyepiece micrometer, the diameter of 150 randomly chosen **microspheres** is determined. (Under 100.times. magnification, 1 division on the micrometer is equal to 1 micron.)

DETD The prior art extraction procedure for production of poly-**lactide**: **glycolide** microencapsulated oral vaccines is based on disbursal of a highly concentrated solution of polymer and acetonitrile into oil, followed by extraction of the acetonitrile and oil with **heptane**.

DETD . . . result, small increases in shear time or minor differences in the emulsifier's rotor dimensions which increased shear, resulted in increased **microsphere** diameters, as can be seen from the graph of FIG. 16.

DETD This result can be seen in Table I, which is in contrast to the data showing **microsphere** volume average versus emulsification time and paraffin oil (FIG. 16).

DETD Reducing the viscosity of the paraffin oil by diluting it with either **heptane** or iso-octane resulted in the formation of a progressively larger spheres as can be seen in Table 2.

DETD . . . 10***

1/2	11.0	4.6	9.7
1/4	2.6	5.0	3.3
1/8	1.5	2.2	2.6
1/16	1.2	1.4	0.6
No Solvent	1.0	1.0	0.6

* = **Heptane** Diluent,
** = Isooctane,
*** = 2nd Series of Isooctane Batches Employing Reduced Shear Forces

DETD The data in Table 1 shows a relationship between the **microsphere** size and oil viscosity in that, **microsphere** size increased as oil viscosity increased from 36 to 65 centistokes and then decreased from 65 to 80 centistokes, which. . .

DETD FIG. 18 shows viscosity versus sphere diameter obtained with paraffin oil diluted with **heptane**.

DETD A histochemical and immunohistochemical analysis of the uptake of **PLG** and polystyrene microparticles by Peyer's patches from a New Zealand white rabbit was conducted using the poly (DL-**lactide** -co-**glycolide**) copolymer in which the molar parts of polymerized **lactide** and **glycolide** were 50:50, as prepared according to the modified solvent extraction process of the invention.

DETD Fluorescent polystyrene **microspheres** were also used as a comparison to test these microparticles as carriers of immunogens for oral immunization, and to ascertain. . .

DETD The study also served in part to ascertain if **encapsulation** may protect the antigens from proteolytic degradation in the gut lumen and facilitate their uptake and retention in the intestinal lymphoid tissues, as a thorough understanding of the fate of ingested **antigen**-containing microparticles is important in using antigens which have been microencapsulated for enteric immunization strategies.

DETD Fluorescent polystyrene **microspheres** and unlabelled poly (**lactide**-co-**glycolide**) **microspheres** of diameters of 0.5, 1, and 2 um where instilled into the lumens of in situ rabbit intestinal loops.

DETD TABLE 3

Microspheres: Particle Size Distribution by Microscopy
between or equal to 5-10 u
Stage magnification: 100 .times.
% volume
% number
Calibration:. . .

DETD TABLE 4

Microspheres: Particle Size Distribution by Microscopy
between or equal to 5-10 u
Stage magnification: 100 .times.
% volume
% number
Calibration:. . .

DETD . . . section of the intestine is that, for oral administration of a vaccine (especially when no booster vaccine is administered), the **antigen** must principally be uptaken by the villous epithelium region, which is more than 90% of the area of the intestine. . .

DETD

IMMUNOHISTOCHEMISTRY
Antibodies:

Monoclonal Antibody	Source	**Antigen** Recognized
V9	Biomeda	Vimentin (M cell marker)

L11-35 Serotec CD43 (pan T cell)
45-3 Spring Valley
MHC11
Ken-4 Spring Valley
CD4
12C7 Spring Valley
. . .
DETD Unexpectedly, **microspheres** also entered non-M cell epithelium cells, especially in the domes. These cells were vimentin negative AcP+. Microparticles were sparse or. . .
CLM What is claimed is:
1. In a solvent extraction process for preparing **microspheres** of an **antigen** containing biodegradable poly(DL-**lactide** -co-**glycolide**), the improvement comprising: preparing a lyophilized **antigen**-sucrose matrix; adding acetonitrile solvent to the **antigen**-sucrose matrix to form a solution; preparing a solution of a biodegradable poly (DL-**lactide**-co-**glycolide**) polymer by adding acetonitrile solvent to the polymer; adding the biodegradable poly (DL-**lactide**-co-**glycolide**)polymer acetonitrile solution to the **antigen** -sucrose acetonitrile solution; adding an oil to the poly (DL-**lactide**-co-**glycolide**)polymer-sucrose-**antigen** solution to form an emulsion having a controlled viscosity, that corresponds to a predetermined average particle size of distributions of **microspheres** of poly (DL-**lactide**-co-**glycolide**) biodegradable polymers of from about 0.5 to about 7.0 micrometers; centrifuging the emulsion of controlled viscosity and removing a supernatant to obtain **microspheres** of the predetermined range of particle size distributions.

2. The process of claim 1, wherein the oil is selected with a predefined viscosity to form the **microspheres**.

6. The process of claim 1, wherein relative ratios between the **lactide** and **glycolide** is 50:50.

L7 ANSWER 3 OF 4 USPATFULL
AB . . . invention further describes a method of immunizing a mammal against diseases comprising administering to a mammal an effective amount of **antigen** containing microparticles. In particular, the present invention describes a method of potentiating an immune response in a mammal comprising administering. . . pharmaceutical composition to a mammal. The present invention further describes a vaccine comprising a pharmaceutical composition containing said microparticles. An **antigen** delivery system comprising microparticles containing entrapped antigens is further described by the present invention. A pharmaceutical composition comprising microparticles and. . .
SUMM . . . further relates to a method of immunizing a mammal against diseases comprising administering to a mammal an effective amount of **antigen** containing microparticles. In particular, the present invention describes a method of potentiating an immune response in a mammal comprising administering. . . said microparticles to a mammal. The present invention further describes a vaccine comprising a pharmaceutical composition containing said microparticles. An **antigen** delivery system comprising microparticles containing entrapped antigens is further described by the present invention. A pharmaceutical composition comprising microparticles and. . .
SUMM Biodegradable polymers such as polylactide-co-**glycolides** (**PLG**) have been used to **encapsulate** proteins and peptides and other drugs for parenteral and/or oral delivery in order to try to achieve a stable and. . . level of drug over an extended period of time. Previous investigators have claimed that antigenic

protein and peptides can be **encapsulated** in microcapsules to deliver "pulses" (i.e. "intermittent doses") of antigenic material for the development of vaccines (see e.g. U.S. Pat.. . .

SUMM The drug release pattern for a microcapsule is dependent upon numerous factors. For example, the type of drug **encapsulated** and the form in which it is present (i.e. liquid or powder) may affect the drugs release pattern. Another factor which may affect the drug release pattern is the type of polymer used to **encapsulate** the drug. Other factors affecting the drug release pattern include the drug loading, the manner of distribution in the polymer,. . .

SUMM . . . solvent. A non-solvent is then added to the suspension or emulsion, causing the polymer to separate from solution and to **encapsulate** the suspended drug particles or droplets containing them. The resulting microparticles (which are still swollen with solvent) are then normally. . .

SUMM Despite numerous modifications to the process of polylactide-co-**glycolides** microparticle formation by phase separation, several problems are usually encountered when following the described techniques of microencapsulation. Such problems include:. . .

SUMM . . . response, leaving an individual move susceptible to diseases at one time point compared to another. Traditional immunization regimens provide an **antigen** to the immune system in discrete pulses. Previous investigators have attempted to convert multiple dose immunization schedules to single dose schedules using controlled release **antigen** delivery systems comprising biodegradable microcapsules. For example, U.S. Pat. No. 5,075,109 to Tice et al. describes a method of immunization in which the **antigen** is delivered in microcapsules of different sizes to attempt to provide an initial dose response followed by a subsequent dose response. The method of Tice attempts to mimic the traditional immunization regime using a single dose of the requisite **antigen**. Although this method alleviates the necessity for providing a booster immunization, this method does not provide a continuous administration of **antigen** and simply provides the traditional burst of **antigen** regimen.

SUMM The theory of providing continuous dose response of an **antigen** to elicit a prolonged immune response was discussed in 1987 by Wise et al. in Advanced Drug Delivery Reviews (1987) 1:19-39. Wise et al. stated that if an **antigen** was released in a continuous manner, the amount of **antigen** presented to the immune system would be too low to induce a protective immune response and may actually lead to tolerance. Recently, Walker in Vaccine (1994) 5:387-400 similarly stated that a sustained release of small amounts of **antigen** over a prolonged time period would likely induce tolerance rather than provide an effective immune response to the **antigen**.

SUMM . . . In particular, contrary to the teachings in the prior art, the present invention provides an essentially continuous release of an **antigen** from microparticles prepared using the novel method described by the present invention. It has been surprisingly discovered in accordance with the present invention that a continuous release of **antigen** results in the induction of immune responses which are comparable to those induced by the potent immunological adjuvant, aluminum hydroxide.

SUMM . . . invention further provides a method of immunizing a mammal against diseases comprising administering to a mammal an effective amount of **antigen** containing microparticles.

SUMM Yet another embodiment of the present invention provides an **antigen** delivery system comprising microparticles containing entrapped antigens.

DRWD FIG. 3 shows that the serum IgG antibody response to OVA in microparticles (OVA/**PLG**) and the response to OVA absorbed to Alum (OVA/Alum) were significantly enhanced in comparison to the response to soluble OVA. . .

DRWD FIG. 4 shows that the serum IgG antibody response to OVA in microparticles (OVA/**PLG**) was significantly enhanced in

comparison to the response to soluble OVA for orally immunized mice.

DETD . . . the first medium is a non-solvent of a pharmacologically-acceptable polymer containing an aqueous solution of the bioactive material to be **encapsulated** (e.g. an aqueous solution of an **antigen**). The second medium is a solvent containing a pharmacologically-acceptable polymer dissolved in the solvent. In a second step to produce. . .

DETD . . . This variation from the prior art leads to a process which provides microparticles of particular value. The material to be **encapsulated** by way of the novel process may be coated with a single wall or "shell" of polymeric material (microcapsules) or may be homogeneously dispersed within a polymeric matrix (**microspheres**). As defined by the present invention, the term microparticles includes both microcapsules and **microspheres** and the term microencapsulation or **encapsulation** should be construed accordingly. The novel process may be used to **encapsulate** a variety of materials.

DETD In accordance with the present invention, the bioactive materials that may be **encapsulated** in microparticles include agricultural agents such as insecticides, fungicides, herbicides, rodenticides, pesticides, fertilizers and viruses for crop protection, as well. . .

DETD . . . media used to produce the microparticles of the present invention will to some extent depend upon the material to be **encapsulated**. When the material to be **encapsulated** in the microparticles is a pharmaceutical agent it is preferably **encapsulated** in a biodegradable polymer. As defined by the present invention a pharmacologically acceptable polymer is biocompatible as well as biodegradable. . . to Casey et al.). Several new biocompatible, biodegradable polymers derived from polyorthoesters and polyorthocarbonates may also be used effectively as **encapsulating** excipients in the practice of the present invention (see, for example, U.S. Pat. Nos. 4,093,709 and 4,138,344 to Choi et. . .

DETD The pharmacologically acceptable polymer preferably used for **encapsulating** the bioactive material of the present invention is a polylactide polymer (PLA), or particularly a polylactide-co-**glycolide** polymer (**PLG**). The ratio of **lactide** to **glycolide** in the most preferred pharmacologically acceptable polymer ultimately determines the rate of release of the bioactive material from the microcapsules,. . .

DETD Generally, the molar ratio of **lactide** to **glycolide** will be between 100:0 and 0:100. In a more preferred embodiment, the molar ratio of **lactide** to **glycolide** will be preferably between 70:30 and 30:70. Thus, a preferred **PLG** polymer has a **lactide:glycolide** ratio of 50:50 and a molecular weight of 9,000 although other polymers which have been used are a **PLG** polymer having a **lactide:glycolide** ratio of 85:15 and a molecular weight of 54,000 and a PLA polymer with a molecular weight of 30,000. It. . . biodegradable polymer or made from different ratios of the same polymer. By utilizing a combination of various polymers with different **lactide/glycolide** ratios, the release profile of the **encapsulated** agent can be controlled.

DETD The **PLG** polymers undergo biodegradation by random, non-enzymatic scission to form the endogenous metabolites lactic acid and glycolic acid. **PLG** microparticles release entrapped pharmaceutical agents as a function of time, by one or more mechanisms, but the release is mainly. . .

DETD Mixed populations of **PLG** microparticles prepared from different polymeric compositions and molecular weights may be engineered to create an essentially continuous release of bioactive. . .

DETD . . . the body. This has advantages in that a single essentially continuous release dose may replace several separate doses of a non-**encapsulated** drug. This may decrease the toxic side effects of

some drugs by avoiding the high initial concentrations of drug in. . .

DETD . . . benzene. In a preferred embodiment, methylene chloride (dichloromethane) and in particular ethyl acetate are the second medium, especially when a **PLG** polymer is used.

DETD . . . may be an alkane or halogenated alkane or a volatile silicone oil. The third medium of the preferred embodiment is **heptane**. The superior surface morphology which may be achieved with the microparticles of the invention may be determined by the measurement. . .

DETD It may be desirable to **encapsulate** bioactive agents for many purposes. Such purposes will govern the pre-use composition of the microparticle. The range of materials which may be **encapsulated**, particularly pharmaceutical agents, is wide and will be apparent to those skilled in the art. U.S. Pat. No. 4,389,330 to. . .

DETD . . . be formulated into various forms of composition depending upon the nature of the material contained therein. Thus, when the microparticles **encapsulate** a pharmaceutical agent, they may be formulated into a pharmaceutical composition together with a physiologically acceptable diluent or carrier for. . .

DETD . . . further directed to a method of immunizing a mammal against disease comprising administering to a mammal an effective amount of **antigen** containing microparticles. In particular, the present invention describes a method of potentiating an immune response in a mammal comprising administering. . .

DETD The present invention is also directed to an **antigen** delivery system comprising the microparticles described by the present invention containing an antigenic material. As used herein, the term antigenic material can include but is not limited to the desired **antigen** peptide, any peptides produced during the synthesis of the desired antigenic peptide, a combination of several desired peptides or the. . .

DETD 100 mg of **antigen** (ovalbumin, hereinafter referred to as OVA) was suspended in 30 g of silicone oil (Dow Corning 200/1000). This was homogenized for 5 minutes with a Silverson homogenizer and then 6 ml of polylactide-co-**glycolide** (**PLG**; 50:50 **lactide** to **glycolide** ratio, m.w. 25,000 daltons) in dichloromethane (2% w/v) was added at a rate of 2 ml per minute. The mixture was homogenized throughout and for a further 1 minute after the addition of the **PLG** solution, cooling with methanol/liquid nitrogen to maintain the temperature at about 12.degree. C. or 22.degree. C. The mixture was then transferred to 300 ml of **heptane** and stirred for 30 minutes. The **heptane** was decanted and an additional 300 ml of **heptane** was added. The mixture was stirred for an additional 30 minutes and then decanted. The microparticles were then washed twice. . .

DETD In a variation of the procedure described above, the 6 ml of 2% w/v **PLG** solution was replaced by either 9 ml of 3% w/v **PLG** solution or 12 ml of 4% w/v **PLG** solution.

DETD . . . a 10% w/w dispersion of the surfactant Span 40 in water was suspended in the silicone oil together with the **antigen** and the whole was homogenized as described before addition of the **PLG**.

DETD . . . BALB/c mice each received primary immunization with 1 mg OVA by gastric intubation on three consecutive days, either as soluble **antigen**, or entrapped in microparticles. Immediately before administration, the required dose of microparticles was resuspended in phosphate buffered saline. Four weeks. . .

DETD . . . shown by proteins (Cohen et al. (1991) Pharmaceutical Research 8:713-720 and Hora et al. (1990) Pharmaceutical Research 7:1190-1194) entrapped in **PLG** microparticles. Studies with an alternative model protein, bovine serum albumin (BSA), entrapped in microparticles have usually shown a typical initial. . . Although microparticle characteristics may be manipulated to minimize the burst effect, it is clear that controlled release systems prepared from **PLG** and

related polymers normally show a release profile incorporating a substantial burst. Therefore, it is o encouraging that the novel. . .

DETD . . . Wise et al. (1987) Walker Advanced Drug Delivery Reviews, 1:19-39; Walker (1994) Vaccine, S:387-400, is that a pulsed profile of **antigen** release is necessary for the induction of potent immune responses and that continuous **antigen** release is more likely to result in the induction of tolerance or o unresponsiveness. The microparticles prepared using the novel method described in the present invention displayed a continuous release of entrapped **antigen** and, contrary to establish teaching in the art, induced an enhanced immune response.

DETD Consequently, the belief that pulsed **antigen** release is required to induce an enhanced immune response is unfounded in light of the surprising findings obtained using microparticles prepared by the novel method as described in the present invention. These microparticles exhibited continuous release of **antigen**, but also induced enhanced antibody responses.

CLM What is claimed is:

3. A method according to claim 1 wherein said bioactive material is an **antigen**.

5. The method according to claim 4 wherein said pharmacologically acceptable polymer is a polylactide-co-**glycolide** or a polylactide.

8. The method according to any of claims 1-5 further comprising a third medium **heptane**.

9. The method according to claim 1 wherein said pharmacologically acceptable polymer is composed of the dimers D,L-**lactide** and **glycolide** in which between 0 to 100% of the polymer is D,L-**lactide** and in which between 0 to 100% of the polymer is **glycolide**.

. . . mammal comprising parenterally administering to said mammal microparticles produced by the process according to claim 1 and which contain an **antigen**.

20. The method according to claim 17 wherein said **antigen** is released in a continuous manner.

26. The vaccine according to claim 25 wherein said microparticles are comprised of a matrix which includes an **antigen** and a biodegradable polymer.

27. The vaccine according to claim 25 wherein said microparticles are comprised of a matrix which includes an **antigen**, a surfactant and a biodegradable polymer.

L7 ANSWER 4 OF 4 USPATFULL

TI Vaccines against diseases caused by enteropathogenic organisms using antigens **encapsulated** within biodegradable-biocompatible **microspheres**

AB . . . invention is directed to oral parenteral and intestinal vaccines and eir use against diseases caused by enteropathogenic organisms using antigens **encapsulated** within biodegradable-biocompatible **microspheres**.

SUMM This invention relates to parenteral and oral-intestinal vaccines against diseases caused by enteropathogenic organisms using antigens **encapsulated** within biodegradable-biocompatible **microspheres** (matrix).

SUMM . . . mucosal surface. Numerous studies have demonstrated that a protective mucosal immune response can best be initiated by introduction

of the **antigen** at the mucosal surface, and parenteral immunization is not an effective method to induce mucosal immunity. **Antigen** taken up by the gut-associated lymphoid tissue (GALT), primarily by the Peyer's patches in mice, stimulates T helper cell (T.sub.H). . . . assist in IgA B cell responses or stimulates T suppressor cells (T.sub.S) to mediate the unresponsiveness of oral tolerance. Particulate **antigen** appears to shift the response towards the (T.sub.H) whereas soluble antigens favor a response by the (T.sub.S). Although studies have demonstrated that oral immunization does induce an intestinal mucosal immune response, large doses of **antigen** are usually required to achieve sufficient local concentrations in the Peyer's patches. Unprotected protein antigens may be degraded or may. . .

SUMM One possible approach to overcoming these problems is to homogeneously disperse the **antigen** of interest within the polymeric matrix of appropriately sized biodegradable, biocompatible **microspheres** that are specifically taken up by GALT. Eldridge et. al. have used a murine model to show that orally-administered 1-10 micrometer **microspheres** consisting of polymerized **lactide** and **glycolide**, (the same materials used in resorbable sutures), were readily taken up into Peyer's patches, and the 1-5 micrometer size were rapidly phagocytized by macrophages. **Microspheres** that were 5-10 micrometers (microns) remained in the Peyer's patch for up to 35 days, whereas those less than 5. . . toxoid and inactivated influenza A virus were enhanced and remained elevated longer in animals which were immunized orally with microencapsulated **antigen** as compared to animals which recieved equal doses of non-**encapsulated** **antigen**. These data indicate that microencapsulation of an **antigen** given orally may enhance the mucosal immune response against enteric pathogens. AF/R1 pili mediate the species-specific binding of E. coli. . .

SUMM . . . of lymphocytes taken from the Peyer's patch, MLN, and spleen of rabbits which have recieved intraduodenal priming with microencapsulated or non-**encapsulatled** AF/R1. Our results demonstrate the microencapsulation of AF/R1 potentiates the cellular immune response at the level of the Peyer's patch,. . .

SUMM This invention relates to a novel pharmaceutical compositon, a microcapsule/sphere formulation, which comprises an **antigen** **encapsulated** within a biodegradable polymeric matrix such as poly (DL-**lactide**-co-**glycolide**) (DL-**PLG**), wherein the relative ratio between the **lactide** and **glycolide** component of the DL-**PLG** is within the range of 52:48 to 0:100, and its use, as a vaccine, in the effective pretreatment of animals. . . the immunogenicity of antigens that contact the intestinal mucosa, applicants investigated the effect of homogeneously dispersing AF/R1 pili within biodegradable **microspheres** that included a size range selected for Peyer's Patch localization. New Zealand White rabbits were primed twice with 50 micrograms. . .

DRWD FIG. 1 shows the size destribution of **microspheres** wherein the particle size distibution (%) is (a) By number 1-5 (91) and 6-10 (9) and Co) By weight 1-5. . .

DRWD FIG. 2 shows a scanning electron micrograph of **microspheres**.

DRWD . . . be immunogenic for rabbit spleen mononuclear cells in vitro producing a primary IgM antibody response specific to AF/RI. Immunization with **antigen encapsulated** in biodegradable, biocompatible **microspheres** consisting of **lactide/glycolide** copolymers has been shown to endow substantially enhanced immunity over immunization with the free **antigen**. To determine if microencapsulated AF/RI maintains the immunogenicity of the free pilus protein, a primary in vitro immunization assay was. . . with free AF/RI in a dose range from 15 to 150 ng/ml or with equivalent doses of AF/RI contained in **microspheres**. Supernatants were harvested on days 7, 9, 12, and

14 of culture and were assayed for free AF/RI pilus protein. . .

DRWD . . . immunogenic for rabbit Peyer's patch mononuclear cells in vitro producing a primary IgM antibody response specific to AF/RI. Immunization with **antigen encapsulated** in biodegradable, biocompatible **microspheres** consisting of **lactide/glycolide** copolymers has been shown to endow substantially enhanced immunity over immunization with the free **antigen**. To determine if microencapsulated AF/RI maintains the immunogencity of the free pilus protein, a primary in vitro immunization assay was. . . with free AF/RI in a dose range from 15 to 150 ng/ml or with equivalent dose of AF/RI contained in **microspheres**. Supernatants were harvested on days 7, 9, 12, and 14 of culture and were assayed for free AF/RI pilus protein. . . positive IgM response on all four days of harvest, with the highest antibody response on day 12 with the highest **antigen** dose. Cells immunized with **encapsulated** pilus protein showed a positive response on day 12 with all three **antigen** doses. In conclusion, AF/RI pilus protein maintains immunogenicity to rabbit Peyer's patch cells immunized in vitro after microencapsulation.

DRWD . . . shows proliferative responses to AF/RI by rabbit Peyer's patch cells. Naive rabbits were primed twice with 50 micrograms of either non-**encapsulated** (rabbits 132 and 133) or microencapsulated (rabbits 134 and 135) AF/RI pili by endoscopic intraduodenal inoculation seven days apart. Seven. . . (p=0.0016), and 135 (p=0.0026). Responses were significantly different between the two groups. Comparison of the best responder in the nonencapsulated **antigen** group (rabbit 133) with the lowest responder in the microencapsulated **antigen** group (rabbit 134) demonstrated an enhanced response when the immunizing **antigen** was microencapsulated (p=0.0034).

DRWD Additionally, FIG. 5 relates to the in vitro lymphocyte proliferation after sensitization of rabbit lymphoid tissues with **encapsulated** or non-**encapsulated** AF/RI pilus adhesion of E. coli strain RDEC-1. The AF/RI adherence factor is a plasmid encoded pilus protein that allows RDEC-1 to attach to rabbit intestinal brush borders. We investigated the immunopotentiating effect of **encapsulating** purified AF/RI into biodegradable non-reactive **microspheres** composed of polymerized **lactide** and **glycolide**, materials used in resorbable sutures. The **microspheres** had a size range of 5-10 microns, a size selected for Peyer's Patch localizaiton, and contained 0.62% protein by weight. NZW rabbits were immunized twice with 50 micrograms of either **encapsulated** or non-**encapsulated** AF/RI by intraduodenal later of non-**encapsulated** AF/RI by intraduodenal inoculation seven days apart. Lymphocyte proliferation in respone to purified AR/RI was conducted in vitro at seven days and showed that **encapsulating** the **antigen** into **microspheres** enhanced the cellular immune response in the Peyer's Patch; however, no significant increase was observed in spleen or mesenteric lymph node. These data suggest that **encapsulation** of AF/RI may potentiate the mucosal cellular immune response.

DRWD . . . responses to AF/RI synthetic peptides by rabbit Peyer's patch cells. Naive rabbits were primed twice with 50 micrograms of either non-**encapsulated** (rabbits 132 and 133) or microencapsulated (rabbits 134 and 135) AF/RI pili by endoscopic intraduodenal inoculation seven days apart. Seven. . . and 108-123 as a T and B cell epitope. We used these peptides to investigate a possible immunopotentiating effect of **encapsulating** purified Af/RI pili into biodegradable, biocompatible **microspheres** composed of polymerized **lactide** and **glycolide** at a size range that promotes localization in the Peyer's Patch (5-10 micrometers). NZW rabbits were primed twice with 50. . . inoculation and their Peyer's Patch cells were cultured in vitro with the AF/RI peptides. In two rabbits which had received **encapsulated** AF/RI, lymphocyte proliferation was observed to AF/RI 40-55 and 79-94 in both rabbits and to 108-123 in one

of two rabbits. No responses to any of the peptides were observed in rabbits which received non-**encapsulated** AF/RI. These data suggest that **encapsulation** of AF/RI may enhance the cellular response to peptide antigens.

DRWD . . . was to determine if AR/R1 pilus protein immune response is enhanced by microencapsulation. The AF/R1 was incorporated into biodegradable, biocompatible **microspheres** composed of **lactide-glycolide** copolymers, had a size range of 5-10 micrometer and containing 0.62% pilus protein by weight. Initially, NZW rabbits were immunized twice with 50 micrograms of either **encapsulated** or non-**encapsulated** AF/RI via intraduodenal route seven days apart. For in vitro challenge, 6.times.10.sup.5 rabbit lymphocytes, were set in microculture at final. . . predicted epitopes were similar to those obtained with purified AF/RI. In conclusion, intestinal immunization with AF/RI pilus protein contained within **microspheres** greatly enhances both the spleen and Peyer's patch B-cell responses to predicted T & B-cell epitopes.

DRWD . . . proliferative responses to AF/R1 by rabbit mesenteric lymph node cells. Naive rabbits were primed twice with 50 ug of either non-**encapsulated** (rabbits 132 and 133) or microencapsulated (rabbits 134 and 135) AF/R1 pili by endoscopic intraduodenal inoculation seven days apart. Seven. . .

DRWD . . . 6.0, 0.6, and 0.06 micrograms/ml. Values shown represent the maximum proliferative response produced by any of the three concentrations of **antigen** used.+-.the standard deviation. The cpm of the control peptide for each of the three monkeys was 1,518.+-.50, 931.+-.28, and 1,553.+-.33. . .

DRWD . . . the proliferative response which occurred to 6.0 micrograms/ml (FIG. 22), 0.6 micrograms/ml (FIG. 23), or 0.06 micrograms/ml (FIG. 24) of **antigen**.+-.the standard deviation. The cpm of the control peptide was 1,553.+-.33 and the cpm of the media control was 1,951.+-.245.

DRWD FIG. 25 shows that rabbits numbers 21 and 22 received intraduodual administration of AF/R1 **microspheres** at doses of AF/R1 of 200 micrograms (ug) on day 0 and 100 ug on day 7, 14, and 21. . .

DRWD FIG. 29. Particle size distribution of CFA/II **microsphere** vaccine Lot L74F2 values are percent frequency of number or volume verses distribution. Particle size (diameter) in microns. 63% by. . .

DRWD FIG. 30. Scanning electron photomicrograph of CFA/II **microsphere** vaccine Lot L7472 standard bar represents 5 um distance.

DRWD FIG. 31. Twenty-two hour CFA/II release study of CFA/II **microsphere** vaccine Lot L7472. Percent cumulative release of CFA/II from three sample: A, 33.12 mgm; B, 29.50 mgm c, 24.20 mgm. . .

DRWD FIG. 32. Serum IgG antibody reponse to CFA/II **microsphere** vaccine Lot L7472 following 2 25 ug protein IM immunization on day 0 in 2 rabbits. Antibody determines on serial. . .

DRWD FIG. 33. Serum IgG antibody response to CFA/II **microsphere** vaccine Lot L7F2 following 2 25 ug protein IM immunizations on day 0 if rabbit 107 & 109. Antibody determined. . .

DRWD . . . (FIG. 34(b)), 83 (FIG. 34(c)), 86 (FIG. 34(d)), and 87 (FIG. 34(e)) immunized intraduodenally with 50 mgm protein of CFA/II **microsphere** vaccine 4 and 7 days earlier. The cells are challenged in vitro with CFA/II or BSA at 500, 50 and. . .

DRWD . . . (FIG. 35(b)), 80 (FIG. 35(c)), 88 (FIG. 35(d)), and 91 (FIG. 35(e)) immunized introduodenally with 50 mgm protein of CFA/II **microspheres** vaccine 14 and 7 days earlier. The cells are challenged in vitro with CFA with CFA/II or BSA at 500,. . .

DRWD . . . (FIG. 36(b)), 83 (FIG. 36(c)), 86 (FIG. 36(d)), and 87 (FIG. 36(e)) immunized intraduodenally with 50 mgm protein of CFA/II **microsphere** vaccine 14 and 7 days earlier. These were cells placed into microculture and tested on day 0, 1, 2, 3, 4 and 5 by ELISPOT for cells secreting antibodies specific for CFA/II **antigen**. The results are expressed as number per 9.times.10.sup.6 spleen cells versus culture day tested.

DRWD . . . and tested on days 0, 1, 2, 3, 4 and 5 by ELISPOT for cells secreting antibodies specific for CFA/II **antigen**. The results are expressed as number per 9.times.10.sup.6 spleen cells versus culture day tested.

DRWD FIG. 39. Hepatitis B surface **antigen** release from 50:50 poly (DL-**lactide**-co-**glycolide**).

DRWD FIGS. 11 and 12 serve to illustrate that inclusion of Escherichia coli pilus **antigen** in **microspheres** enhances cellular immunogenicity.

DRWD . . . infection of rabbits with E. coli RDEC-1. However, induction of an optimal primary mucosal response by enteral vaccination with pilus **antigen** depends on immunogenicity of pilus protein, as well as such factors as its ability to survive gastrointestinal tract (GI) transit and to target immunoresponsive tissue. We tested the effect of incorporating AF/R1 pilus **antigen** into resorbable **microspheres** upon its ability to induce primary mucosal and systemic antibody responses after direct inoculation into the GI tract.

DRWD Rabbits were inoculated with 50 micrograms of AF/R1 pilus **antigen** alone or incorporated into uniformaly sized (5-10 microns) resorbably **microspheres** (MIC) of poly(DL-**lactide**-coglycolide). Inoculation was by intra-duodenal (ID) intubation via endoscopy or directly into the ileum near a Peyer's patch via the RITARD procedure (with the cecum ligated to enhance recovery of gut secretions and a reversible ileal tie to slow **antigen** clearance). ID rabbits were sacrificed at 2 weeks for collection of gut washes and serum. RITARD rabbits were bled and. . .

DRWD Native pilus **antigen** led to a mucosal IgA resposne in 7/8 RITARD rabbits. MIC caused a similar response in only 4/8, but the. . .

DRWD Inoculation with pilus **antigen** produces a primary mucosal IgA response. Microencapsulation does not enhance this response, although the **antigen** remains immunogenic as shown by measurable mucosal and some strong serum responses. It must be determined whether priming with **antigen** in **microspheres** can enhance secondary responses.

DRWD . . . pili were purified from H10407 (078:H-) as described by Hall et al, (1989) [20]. Briefly, bacteria grown on colonization factor **antigen** agar were subjected to shearing, with the shearate subjected to differential centrifugation and isopycnic banding on cesium chloride in the. . .

DRWD . . . the pins was chosen by initial titration of sera by standard ELISA assay and immunodot blot assay against the same **antigen**.

DRWD **Antigen**. CFA/I pili were purified from E. coli strain H107407 (serotype 078:H11) by ammonium sulfate precipitation using the method of Isaacson. . .

DRWD . . . had been emulsified in Complete Freund's Adjuvant, by single i.m. injection (0.5 ml). For each animal, the initial dose of **antigen** was followed by two similar injections in Incomplete Freund's Adjuvant at seven day intervals.

DRWD Lymphocyte proliferation. At day 10-14 following the final inoculation of **antigen**, the monkeys were again sedated with ketamine HCl, and 50 ml of blood was drawn from the femoral artery for. . . ml) were plated in 96-well flat bottom culture plates (Costar, Cambridge, Mass.) along with 0.05 ml of various dilutions of **antigen** in cDMEM without serum (yielding a 0.5% final concentration of autologous serum) and were incubated at 37 degrees C. in. . .

DRWD . . . their splenic lymphocytes were cultured with synthetic overlapping decapeptides which represented the entire CF/I sequence. Concentrations of peptides used as **antigen** were 6.0, 0.6, and 0.6 ug/ml. Proliferative responses to the decapeptides were observed in each of the three monkeys (FIGS. 1-3). The majority of the responses occurred at the 0.6 and 0.06 ug/ml concentrations of **antigen** and within distinct regions of the protein (peptides beginning with residues 8-40, 70-80, and 27-137). A comparison of the responses. . .

and 0.06 ug/ml concentrations antigenic peptide for one monkey (2&2) are shown (FIGS. 4-6). Taking into account all concentrations of **antigen** tested, spleen cells from monkey 184D demonstrated a statistically significant response to decapeptides beginning with CFA/I amino acid residues 3,. . .

DRWD Applicants have discovered efficacious pharmaceutical compositions wherein the relative amounts of **antigen** to the polymeric matrix are within the ranges of 0. 1 to 1.5% **antigen** (core loading) and 99.9 to 98.5% polymer, respectively. It is preferred that the relative ratio between the **lactide** and **glycolide** component of the poly(DL-**lactide**-co-**glycolide**) (DL-**PLG**) is within the range of 52:48 to 0: 100. However, it is understood that effective core loads for certain antigens. . . its microscopic form (i.e. bacteria, protozoa, viruses or fungi) and type of infection being prevented. From a biological perspective, the DL-**PLG** or **glycolide** monomer excipient are well suited for in vitro drug (**antigen**) release because they elicit a minimal inflamatory response, are biologically compatible, and degrades under physiologic conditions to products that are. . .

DRWD . . . by enteropathogenic organisms comprising administering orally to said animal an immunogenic amount of a pharmaceutical composition consisting essentially of an **antigen encapsulated** within a biodegradable polymeric matrix. When the polymeric matrix is DL-**PLG**, the most preferred relative ratio between the **lactide** and **glycolide** component is within the range of 48:52 to 52:48. The bacterial infection can be caused by bacteria (including any derivative. . .

DRWD A. (1) To homogeneously disperse antigens of enteropathic organisms within the polymeric matrix of biocompatible and biodegradable **microspheres**, 1 nanogram (ng) to 12 microns in diameter, utilizing equal molar parts of polymerized **lactide** and **glycolide** (50:50 DL-**PLG**, i.e. 48:52 to 52:48 DL-**PLG**) such that the core load is within the range of about 0.1 to 1.5% by volume. The **microspheres** containing the dispered **antigen** can then be used to immunize the intestine to produce a humoral immune response composed of secretory antibody, serum antibody and a cellular immune response consisting of specific T-cells and B-cells. The immune response is directed against the dispered **antigen** and will give protective immunity against the pathogenic organism from which the **antigen** was derived.

DRWD . . . thus promoting colonization resulting in diarrhea. AF/R1 pilus protein was homogeneously dispered within a polymeric matrix of biocompatible and biodegradable **microspheres**, 1-12 microns in diameter (FIG. 1 and photograph 1) using equal molar parts of polymerized **lactide** and **glycolide** (50:50 DL-**PLG**) such that the core load was 0.62% by weight.

DRWD (3) The **microspheres** were found to contain immunogenic AF/R1 by immunizing both rabbit spleen (FIG. 2) and Peyer's patch (FIG. 3) B-cells in. . .

DRWD (4) **Microspheres** containing 50 micrograms of AF/R1 were used to intraintestinally (intraduodenally) immunize rabbits on two separate occasions 1 week apart. One. . .

DRWD B. **Microspheres** do not have to be made up just prior to use as with liposomes. Also liposomes have not been effective. . .

DRWD C. (1) Only a small amount of **antigen** is required (ugs) when dispersed within **microspheres** compared to larger amounts (mgms) when **antigen** is used alone for intestinal immunization.

DRWD (2) **Antigen** dispersed within **microspheres** can be used orally for intestinal immunization whereas **antigen** alone used orally even with gastric acid neutralization requires a large amount of **antigen** and may not be effective for intestinal immunization.

DRWD (3) Synthetic peptides with and without attached synthetic adjuvants representing peptide fragments of protein antigens can also be dispersed

within **microspheres** for oral-intestinal immunization. Free peptides would be destroyed by digestive processes at the level of the stomach and intestine. Any. . .

DRWD (4) **Microspheres** containing **antigen** maybe placed into gelatin-like capsules for oral administration and intestinal release for improved intestinal immunization.

DRWD (5) **Microspheres** promote **antigen** uptake from the intestine and the development of cellular immune (T-cell and B-Cell) responses to **antigen** components such as linear peptide fragments of protein antigens.

DRWD (6) The development of intestinal T-cell responses to antigens dispersed within **microspheres** indicate that T-cell immunological memory will be established leading to long-lived intestinal immunity. This long-lived intestinal immunity (T-cell) is very. . .

DRWD (2) **Microspheres** containing adherence pilus protein AF/R1 or its **antigen** peptides for oral intestinal immunization of rabbits against RDEC-1 infection.

DRWD (4) **Microspheres** containing adherence pilus proteins CFA/I, II, III and IV or their **antigen** peptides for oral intestinal immunization of humans against human enterotoxigenic E. coli infections.

DRWD (2) By using the **microspheres**, we are now able to immunize the intestine of animals and man with antigens not normally immunogenic for the intestinal. . .

DRWD (3) Establishing long-lived immunological memory in the intestine is now possible because T-cells are immunized using **microspheres**.

DRWD (4) Antigens that can be dispersed into **microspheres** for intestinal immunization include the following: proteins, glycoporteins, synthetic peptides, carbohydrates, synthetic polysaccharides, lipids, glycolipids, lipoopolysaccharides (LPS), synthetic lipopolysaccharides and. . .

DRWD . . . can be directed to either systemic (spleen and serum antibody) or local (intestine, Peyer's patch) by the size of the **microspheres** used for the intestinal immunization. **Microspheres** 5-10 microns in diameter remain within macrophage cells at the level of the Peyer's patch in the intestine and lead to a local intestinal immune response. **Microspheres** 1 ng--5 microns in diameter leave the Peyer's patch contained within macrophages and migrate to the mesenteric lymph node and. . .

DRWD . . . antibody mediated adverse reactions because of preexisting antibody especially cytophyllic or IgE antibody may be minimized or eliminated by using **microspheres** because of their being phagocytized by macrophages and the **antigen** is only available as being attached to the cell surface and not free. Only the free **antigen** could become attached to specific IgE antibody bound to the surface of mast cells resulting in mast cell release of. . .

DRWD (7) Immunization with **microspheres** containing **antigen** leads to primarily IgA and IgG antibody responses rather than an IgE antibody response, thus preventing subsequent adverse IgE antibody reactions upon reexposure to the **antigen**.

DRWD In addition to the above, the **encapsulation** of the following synthetic peptides are contemplated and considered to be well within the scope of this invention:

DRWD The profile of the representative experiments have been chosen to illustrate the effectiveness of the immunogenic polymeric matrix-**antigen** composites.

DRWD . . . be greater than 95% pure by scanning with laser densitometry when stained with coomassie blue. Briefly, equal molar parts of DL-**lactide** and **glycolide** were polymerized and then dissolved to incorporate AF/R1 into spherical particles. The **microspheres** contained 0.62% protein by weight and ranged in size from 1 to 12 micrometers. Both the microencapsulated and non-**encapsulated** AF/R1 were sterilized by gamma irradiation (0.3 megarads) before use.

DRWD Immunization. Rabbits were primed twice with 50 micrograms of either

microencapsulated or non-**encapsulated** AF/R1 by endoscopic intraduodenal inoculation seven days apart by the following technique. All animals were fasted overnight and sedated with. . . inserted through the biopsy channel and threaded 2-3 cm into the small intestine. Inoculums of pili or pili embedded in **microspheres** were injected through the catheter into the duodenum and the endoscope was withdrawn. Animals were monitored daily for signs of. . .

DRWD . . . ml) were placed in 96-well flat bottom culture plates (Costar, Cambridge, Mass.) along with 0.1 ml of various dilutions of **antigen** and were incubated at 37.degree. C. in 5% CO.sub.2. In other experiments, cultures were conducted in a 24-well plates. In these experiments, 5.times.10.sup.6 cells were cultured with or without **antigen** in a 2 ml volume. After 4 days, 100 microliters aliquots of cells were transferred to 96-well plates for pulsing and harvesting. Previous experiments have demonstrated that optimal concentrations of **antigen** range from 150 ng/ml to 15 micrograms/ml in the 96-well plate assay and 1.5 ng/ml to 150 ng/ml in the. . .

DRWD . . . shown as a stimulation index (SI) to facilitate the comparison. SI were calculated by dividing the mean of cultures with **antigen** by the mean of cultures without **antigen** (media control). Statistical significance (p value) was determined by comparing the maximum response for each **antigen** to the media control using the Student's t test.

DRWD . . . small, but significant proliferation of the spleen cells to all the AF/R1 peptides tested as compared to cell cultures without **antigen** (FIG. 14). Cells from the spleen and Peyer's patches of non-immune animals failed to respond to either AF/R1 or the. . .

DRWD . . . immune response. To evaluate the effect that microencapsulation of AF/R1 may have on the cellular mucosal immune response to that **antigen**, naive rabbits were primed twice with 50 micrograms of either microencapsulated or non-**encapsulated** AF/R1 by endoscopic intraduodenal inoculation seven days apart. All rabbits were monitored daily and showed no evidence of clinical illness. . . sacrificed and lymphoid tissues were cultured in the presence of AF/R1 pili or peptide antigens. In rabbits which had received non-**encapsulated** AF/RI, Peyer's Patch cells demonstrated a low level but significant proliferation in vitro in response to AF/R1 pili (FIG. 5),. . .

DRWD . . . a significant proliferation in vitro in response to AF/R1 pili regardless of whether they had been immunized with microencapsulated or non-**encapsulated** AF/R1 (FIG. 15). However, only the rabbits which had received microencapsulated AF/R1 were able to respond to the AF/R1 synthetic. . .

DRWD . . . in vitro proliferative response to both protein and its peptide antigens by rabbit Peyer's patch cells following intraduodenal inoculation of **antigen** which had been homogeneously dispersed into the polymeric matrix of biodegradable, biocompatible **microspheres**. The immunopotentiating effect of **encapsulating** purified AF/R1 pili as a mucosal delivery system may be explained by one or more of the following mechanisms: (a) Microencapsulation may help to protect the **antigen** from degradation by digestive enzymes in the intestinal lumen. (b) Microencapsulation has been found to effectively enhance the delivery of a high concentration of **antigen** specifically into the Peyer's patch. (c) Once inside the Peyer's patch, microencapsulation appears to facilitate the rapid phagocytosis of the **antigen** by macrophages, and the **microspheres** which are 5-10 micrometers become localized within the Peyer's patch. (d) Microencapsulation of the **antigen** may improve the efficiency of **antigen** presenttion by decreasing the amount of enzymatic degradation that takes place inside the macrophage before the epitopes are protected by combining with Class II major histocompatibility complex (MHC) molecules. (e) The slow, controlled-release of **antigen** may produce a depot effect that mimics the retention of **antigen** by

the follicular dendritic cell. (f) If the **antigen** of interest is soluble, microencapsulation changes the **antigen** into a particulate form which appears to assist in producing an IgA B cell response by shifting the cellular immune. . . the GALT may be able to discriminate between microbial and non-microbial (food) antigens in part by the form of the **antigen** when it is first encountered, and thus bacterial antigens do not necessarily have special antigenic characteristics that make them different. . . food antigens, but they are antigenic because of the bacterial context in which they are presented. The particulate nature of **microspheres** may serve to mimic that context. It may be important to note that we also observed a significant response to AF/R1 in animals inoculated with non-**encapsulated** pili; thus, some of this **antigen** which was still in its native form was able to enter the Peyer's patch. This may be explained by the fact that AF/R1 is known to mediate the attachment of RDEC-1 to the Peyer's patch M-cell. If the **antigen** employed in this type of study was not able to attach to micrometer M-cells, one would expect to see an even greater difference in the responses of animals which had received microencapsulated versus non-**encapsulated antigen**.

DRWD The **microspheres** used in these experiments included a size range from 1 to 12 micrometers. The 1 to 5 micrometer particles have. . . the observed proliferative responses by cells from the MLN and spleen may reflect priming of MLN or splenic lymphocytes by **antigen** -presenting/accessory cells which have phagocytosed 1 to 5 micrometer **antigen**-laden **microspheres** in the Peyer's patch and then disseminated onto the MLN. Alternatively, these responses may be a result of the normal migration of **antigen** stimulated lymphocytes that occurs from the Peyer's patch to the MLN and on into the general circulation before homing to. . .

DRWD The proliferative response to the peptide antigens was of particular interest in these studies. The rabbits that received non-**encapsulated** AF/R1 failed to respond to any of the peptides tested either at the level of the Peyer's patch, the MLN,. . . to varing kinetics of sensitized T cell migration in different rabbits, or they may reflect differences in the efficiency of **antigen** presentation by cells from different lymphoid tissues of these animals. Of all the synthetic peptides tested, only AF/R1 40-55, (the. . . the amino acid sequence of this peptide includes an immunodominant B cell epitope. Thus AF/R1 40-55 may readily bind to **antigen**-specific B cells thereby leading to an efficient B cell presentation of this **antigen** to sensitized T cells. Even though AF/R1 40-55 was not selected as a probable T cell epitope by either the. . .

DRWD . . . the kinetics of cellular migration. The rabbits in this study were sacrificed only two weeks after their first exposure to **antigen**. This relatively short time period may not have provided sufficient time for cells that were produced by Peyer's patch and. . .

DRWD . . . be effective without requiring carder molecules or adjuvants which may complicate vaccine production or delay regulatory approval. The incorporation of **antigen** into **microspheres** appears to provide an ideal mucosal delivery system for oral vaccine immunogens because the observed immunopotentiating effect is achieved without. . .

DRWD . . . intitiate a mucosal response but is susceptiple to digestion in the gut. The incorporation of AF/R 1 into biocompabible, nondigestible **microspheres** enhanced mucosal cellular immune respones to RDEC-1. We have demonstrated that immunization with AF/R1 Pili in **microspheres** protect rabbits against infection with RDEC-1.

DRWD Six rabbits received intra-duodenal immunization of AF/R1 **microspheres** (0.62% coreloading by weight) at 200 ug AF/R1 on day 0 then boosted with 100 ug AF/R1 in **microspheres** on days 7, 14, and 21 followed RDEC-1 challenge with 10.sup.8 organisms one week latter than observed for 1 week. . . infection and strongly indicates similiar results should be expected with entertoxigenicity E. coli using

the Colony Forming Antigens (CFA's) in **microsphere** vaccines.

DRWD . . . we showed potentiation of the mucosal cellular immune response to the AF/R1 pilus of RDEC-1 by incorporation into biodegradable polylactide-coglycolide **microspheres** (AF/R1-MS). We now present efficacy testing of this vaccine. Six rabbits were primed with 200 ug and boosted with 100. . .

DRWD More recently, applicants have focused on areas of this invention related to an immunostimulating composition comprising **encapsulating microspheres**, which may contain a pharmaceutically-acceptable adjuvant, wherein said **microspheres** are comprised of (a) a biodegradable-biocompatible poly (DL-**lactide**-co-**glycolide**) as the bulk matrix, wherein the relative ratio between the amount of **lactide** and **glycolide** components are within the range of 52:48 to 0:100 and (b) an immunogenic substance comprising Colony Factor **Antigen** (CFA/II, hepatitis B surface **antigen** (HBsAg), or a physiologically similar **antigen** that serves to elicit the production of antibodies in mammalian subjects.

DRWD 1. An immunostimulating composition comprising **encapsulating-microspheres**, which may contain a pharmaceutically-acceptable adjuvant, wherein said **microspheres** having a diameter between 1 nanometers (nm) to 10 microns (um) are comprised of (a) a biodegradable-biocompatible poly (DL-**lactide**-co-**glycolide**) as the bulk matrix, wherein the relative ratio between the amount of **lactide**: and **glycolide** components are within the range of 52:48 to 0:100 and (b) an immunogenic substance comprising Colonization Factor **Antigen**, hepatitis B surface **antigen** (HBsAg), or a physiologically similar **antigen** that serves to elicit the production of antibodies in mammalian subjects.

DRWD 3. An immunostimulating composition according to paragraph 2 wherein the relative ratio between the **lactide** and **glycolide** component is within the range of 48:52 to 52:48.

DRWD 4. An immunostimulating composition according to paragraph 2 wherein the size of more than 50% of said **microspheres** is between 5 to 10 um in diameter by volume.

DRWD 6. A vaccine comprising an immunostimulating composition of paragraph 5 wherein said immunogenic substance is Colony Factor **Antigen** (CFA/II).

DRWD 7. A vaccine comprising an immunostimulating composition of paragraph 5 wherein said immunogenic substance is hepatitis B surface **antigen** (HBsAg).

DRWD In sum, the Colony Factor **Antigen** (CFA/II) from enterotoxigenic E coli (ETEC) prepared under GMP was successfully incorporated into biodegradable polymer **microspheres** (CFA/II BPM) under GMP and found to be safe and immunogenic when administered intra-duodenally to rabbits. CFA/II was incorporated into poly (D,L-**lactide**-co-**glycolide**) (PLGA) **microspheres** which were administered by direct endoscopy into the duodenum. Following vaccination, Peyer's patchcells responded by lymphocyte proliferation to in vitro. . . CFA/II BPM contained 63% between 5-10 um by volume particle size distribution; 1.17% protein content; 2.15% moisture; <0.01% acetonitrile; 1.6% **heptane**; 22 nonpathogenic bacteria and 3 fungi per 1 mgm protein dose; and passed the general safety test. We conclude that. . .

DRWD . . . first step in pathogenesis is adherence to the small intestine epithelial cells by protein fimbrial (pilus) adhesins called colonization factor **antigen** (CFA). Three major CFAs have been recognized, CFA/I, CFA/II and CFA/IV. (25)

DRWD D and L-lactic acid and glycolic acid, as homo- and copolymers, are biodegradable and permit slow and continued release of **antigen** with a resultant adjuvant activity. These polymers have been shown to be safe in a variety of applications in human beings and in animals (28-32). Delivery of antigens via **microspheres** composed of

biodegradable, biocompatible **lactide/glycolide** polymers (29-32) may enhance the mucosal response be protecting the **antigen** from digestion and targeting them to lymphoid cells in Peyer's patches (29-32). McQueen et al. (33) have shown that E. coli AF/R1 pili in PLGA **microspheres**, introduced intra-duodenally in rabbits, protected them against diarrhea and weight loss when challenged with the parent strain rabbit diarrheagenic strain. . .

DRWD In order to improve the CFA/II vaccine it was incorporated into PLGA **microspheres** under GMP in order to protect it from digestion and target it to the intestinal lymphoid system. The CFA/II BPM. . .

DRWD . . . (about 90-120 minutes). Sterile water was added to each tube to disperse the CFA/II retained on the filter. The desalted **antigen** dispersions from all tube were pooled and then divided into five equal parts by weight so as to contain 20 mg of the CFA/II each. The desalted **antigen** dispersion was stored at -10.degree. to -20.degree. C.

DRWD CFA/II Biodegradable Polymer **Microspheres**

DRWD About 1 mgm of **microspheres** were dispersed in 2 ml of 1% Polysorbate 60.degree. (Ruger Chemical Co. Inc. Irvington, N.J.) in water in a 5. . . observed under a calibrated optical microscope with 43.times. magification. Using a precalibrated eye-piece micrometer, the diameter of 150 randomly chosen **microspheres**, was determined and the **microsphere** size distribution was determined

DRWD **Microspheres** were sprinkled or the surface of 10 mm stub covered with a non-conductive adhesive (Sticky-Tab, Ernest F. Fullem, Inc., Lutham,. . .

DRWD Preparation Of CFA/II **Microspheres**

DRWD Solvent extraction techique was used to **encapsulate** the freeze dried CFA/II into poly(**lactide**-co-**glycolide**)(Medisorb Techologies International, visocity 0.73 dl/g) **microspheres** in the 1-10 um size range to achieve theoretical **antigen** loading of 1% by weight. The freeze dried **antigen**-sugar & matrix was dispersed in an acetolnitrile solution of the polymer and then emulsified to achieve desired droplet size. **Microspheres** were solidified and recovered by using **heptane** as extracting solvent. The **microsphere** batches were pooled and vacuum dried to remove traces of solvent.

DRWD Protein Content The CFA/II **microspheres** were dissolved in 0.9% SDS in 0.1N NaOH for 18 hr with stirring then neutralized to pH 7 and assayed.. . .

DRWD One hundred and fifty mgm of CFA/II **microspheres** were dissolved in 3 ml of acetonitrile by sonication for 3 hours. One ml sample was injected into a Karl. . .

DRWD Acetonitrile and **Heptane** Residuals

DRWD Ten mgm of CFA II **microspheres** were dissolved in 1 ml DMF then analysed using gas chromatography and comparing peak heights to external standards of either acetonrile or **heptane** diluted in DMF with 10 mgm of blank **microspheres**. The results are expressed as percent by weight.

DRWD One hundred mgm of CFA/II **microsphere**(single dose) are suspended in 2 ml of sterile saline than poured into 2 blood agar plates (1 ml each). All. . . are counted and identified after 48 hours in culture at 37.degree. C. and expressed as total number. Similiar amount of **microspheres** is in 0.25 ml aliquots poured onto 4 different fungal culture plates (Sabhiragar, casein peptone agar with chloramphenicol, brain heart. . .

DRWD CFA/II Release From **Microsphere** Study

DRWD Two doses of one hundred mgm CFA/II **microspheres** were suspended by sonication for 5 minutes in 3.1 mls of sterile vaccine dilutent consisting of injectable saline containing 0.5%. . .

DRWD Two Rabbits were immunized with CFA/II **microsphere** vaccine at 25 ug protein in two different sites intra-muscularly on day 0. Sera were obtained from all animals before. . . immunization on day o and days 7 and 14. The sera were tested by ELISA for IgG antibodies to CFA/II **antigen** and individual coli surface (CS) proteins CS3

and CS 1. ELISA plates were coated with 3 ug/ml of either CFA/II **antigen**, CS3 or CS1 protein (150 ul/well) and incubated with 150 ul/well of PBS with 0.1% BSA for four hours at. . .

DRWD Rabbits (N=5) were vaccinated with CFA/II **microspheres** containing either 25 or 50 ug of protein suspended in 1 ml of PBS containing 0.5% Polysorbrate 60.RTM. on day 0 and 7 by sonication. The **microspheres** were injected through an Olympus BF type P10 bronchoscope into the duodenum of the rabbits following sedation with an intra. . . catheter passed through the biospy channel. The catheter was advanced through the pylorus 3-4 cm into the duodemum and the **microsphere** suspension in 1 ml of PBS was injected, followed by a 9 ml flush of PBS and removal of the. . .

DRWD . . . 2.5.times.10.sup.6 cells/ml for each well of a 24 well plate. These cells were challenged separately with BSA and the CFA/II **antigen** at doses of 500, 50 and 5 ng/ml in triplicate wells. The plates were incubated at 37.degree. C. with 5%. . . were transferred into each of 4 wells in a 96 well flat bottom microculture plate. Thus, the challenge at each **antigen** dose represented by 3 wells in the 24 well plate is now represented by 12 wells in the 96 well. . .

DRWD Spleen cells were obtained from immunized rabbits on day 14 following intra-duodenal immunization with CFA/II **microsphere** vaccine. The cells were placed in 96 well round bottom microculture plate at a final concentration of 6.times.10.sup.5 cells/well and. . . days at 37.degree. C. with 5 CO.sub.2. 96 well flat bottom microculture plates were coated with 3 ug/ml of CFA/II **antigen** overnight blocked with PBS with 0.05% Polysorbate 60.RTM.. On the harvest days, the cells were gently flushed out of the wells of the round bottom plates and transferred to the corresponding well in the **antigen** coated, 96 well flat bottom microculture plates to be tested for the presence of antibody secreting cells using ELISPOT technique.. . .

DRWD The results of size frequency analysis of 150 randomly chosen **microspheres** are shown in (FIG. 29). The particle size distribution is plotted in % frequency against particle size in diameter (size). . .

DRWD The **microspheres** are seen in (FIG. 30) which is a scanning electron photomicrograph. Nearly all the **microspheres** are less than 10 um as compared to the 5 um bar. Also the surfaces of the **microsphere** are smooth and demonstrate lack of pores.

DRWD . . . 1.232%.+-.0.13 SD; and K65A8, 0.966%.+-.0.128 SD. The mean average protein load is 1.16%.+-.0.15 SD. The protein load of the CFA/II **microsphere** vaccine in the final dose vial is the following: Lot L74F2, 1.175%.+-.0.17SD.

DRWD The CFA/II **microsphere** vaccine (Lot 74F2) percent water content was found using the Karl Fischer titrimeter method to be 2.154% using triplicate samples.

DRWD Acetonitrile and **Heptane** Residuals

DRWD The acetonitrile residuals of the 4 individual CFA/II **microsphere** batches are the following: K62A8, <0.1%; K62A8, <0.1%, K64A8, <0.1%; and K65A8, <0.1%. The acetonitrile residual of the CFA/II **microsphere** vaccine in the final dose vial is the following: Lot L74F2, 0.07.+-.0.05%. The **heptane** residual of the 4 individual CFA/II **microsphere** batches are the following:K62A8, 1.9%; K63A8, 1.4%; K64A8, 1.6% and K65A8, 1.6%. Following pooling in **heptane** and subsequent drying, the **heptane** residual of the CFA/II **microsphere** vaccine in the final dose vial is the following: Lot L74F2, 1.6.+-.0.1%.

DRWD One hundred milligrams (a single dose) of CFA/II **microsphere** vaccine (Lot L74F2) in the final dose vial was suspended in a 2 ml of sterile saline and 1 ml. . . as a micrococus species. All these bacteria are considered to be nonpathogenic to humans. An additional 100 mgms of CFA/II **microsphere** vaccine (Lot L74F2) were suspended in 2 ml of sterile saline and 0.25 ml poured onto four different fungal culture. . .

DRWD CFA Release From **Microsphere** Study

DRWD Two one hundred milligrams(a single dose) of CFA/II **microsphere** vaccine in the final dose vials were suspended in 3.1 mls of the sterile dilulent consisting of 0.85N saline prepared. . .

DRWD . . . The mice gained and average of 2.3 gms and the guinea pigs gained and average, of 43 grams. The CFA/II **microsphere** vacccine therefore passed the general safety test.

DRWD Two rabbits were immunized in two separate sites intra-muscularly with 25 ug of protein of CFA/II **microsphere** vaccine (Lot L74F2) in the final dose vial. Sera samples were obtained before and 7 and 14 days following immunization.. . .

DRWD Five rabbits were immunized intra-duodenally with CFA/II **microspheres** containing either 25 ug of protein (human dose equivalent) or 50 ug of protein on days 0 and 7 and then sacrificied on day 14. The Peyer's patch lymphocytes were challenged in vitro with CFA/II **antigen**, BSA media and alone. The lymphocyte transformation was determined by tritriated thymidine incorporation. The results of the high dose immunization are seen in (FIG. 34). The results are expressed as Kcpm against **antigen** dose. No response to BSA or media control is seen in any of the five rabbits. All rabbits responded by. . .

DRWD Five rabbits immunized intraduodenally with CFA/II **microsphere** containing 50 ug of CFA/II protein at days 0, 7 than scarified at day 14 were studied. The spleen cells. . .

DRWD McQueen et al (33)has found that the AF/R1 adhesin of rabbit diarrheagenic Escheria coli (RDEC-1) incorporated into biodegrable **microspheres** could function as a safe and effective oral intestinal vaccine in the rabbit diarrhea model. The AF/R1 was incorporated into poly D,L-**lactide**-co-**glycolide**) **microspheres** and administered intraduodenally. Jarboe et al (34) reported that

DRWD Peyer's patch cells obtained from rabbits immunized intra-duodenually with AF/R1 in **microspheres** responded with lymphocte proliferation upon in vitro challenge with AF/R1. This early response at 14 days gave a clear indication as to the immunogenicity of E. coli pili contained within the polymer **microspheres**.

DRWD The CFA/II vaccine has now been incorporated into Poly(D,L **lactide**-co-**glycolide**) **microspheres** under Good Manufacturing Practices and tested under Good Laboratory Practices. The **microspheres**, are spherical, smooth surfaced and without pores. The majority (63%) are between 5-10 um in diameter by volume. This. . . was the goal of the vaccine formulation. One percent was chosen because 0.62% was the core loading of the AF/R1 **microspheres** which were effective. Also a small precentage perhaps 1-5% (35) is anticipated to be taken up from the intestine, a. . .

DRWD The organic residuals are of course a concern. **Heptane** exposure would be 1.7 mgm per vaccine dose. This is compared to the occupational maximum allowable exposure of 1800 mgm/15 min. Therefore, the **heptane** contained with the CFA/II **microsphere** vaccine appears to be a safe level. The acetonitrile is very low -0.1 mgm per vaccine dose. The human oral TDLO is 570 mgm/Kg (any non letheal toxicity). Therefore, the acetonitrile contained with the CFA/II **microsphere** vaccine appears to be at a safe level. The CFA/II vaccine was produced under sterile conditions. However, the process of incorporation of the desalted CFA/II vaccine into the polymer **microsphere** batches and subsequent pooling and loading final dose vials was done in a clean room as for any oral medication.. . . Ty 21 a oral). Two hundred non pathogenic bacteria are allowed as well as 20 fungi per dose. The CFA/II **microsphere** vaccine is well under these requirements having only 22 non-pathogenic bacteria and 3 fungi per dose.

DRWD . . . general safety test was also patterned after the WHO requiremets for the TY, 21a oral vaccine in that the CFA/II **microsphere** vaccine was give by gastric lavage to the guinea

pigs. Both mice and both guinea pigs demonstrated no toxicity &. . .
DRWD The CFA/II **microsphere** vaccine (Lot74F2) is immunogenic giving high titer serum IgG antibody responses as early as 7 days following intra muscular injection in rabbits. This test will be used as potency test for future lots of the CFA/II **microsphere** vaccine. Slighly higher antibody titers were seen towards the CS3 pilus protein and this may reflect that CS3 accounts for. . .
DRWD The CFA/II **microsphere** vaccine was also immunogenic following intra-duodenal administration to rabbits. The highest lymphocyte proliferative responses from Peyer's patch cells were seen with the lower 25 ug dose. This is the human equivalent dose and suggests that higher doses of **antigen** in polymer **microspheres** may attenuate, this immunological reponse.
DRWD Further evidence of immunization by the CFA/II **microsphere** vaccine given intra-duodenually is demonstrated by the lymphatic hyperplasia in the spleen seen to a greater extend in the rabbits. . .
DRWD . . . microencapsulation were studied to determine what criteria must be satisfied to provide a protective immune response to hepatitis B surface **antigen** (HBsAg) after a single injection of vaccine. In mouse studies, the 50% effective dose (ED.sub.50) for the alum precipitated Heptavax. . . was 3.8 ng when administered in a 3 injection regimen, but was 130 ng when one immunizing dose was used. **Antigen** release studies revealed that HBsAg is bound tightly to the alum, indicating that the **antigen** remains in situ until scavenged by phagocytic cells. the ED.sub.50 with a 3 dose regimen of aqueous HBsAg was 180. . . day intervals over 90 days. The ED.sub.50 was 220 ng for a single dose regimen of HBsAg microencapsulated in poly (DL-**lactide**-co-**glycolide**) in a form that was too large to be phagocytized and had an **antigen** release profile similar to that achieved with the geometrically decreasing regimen of doses. This indicates that single injection of microencapsulated. . .
DRWD . . . the body, multiple doses and boosters are usually required for continued protection.sup.37. Alum adjuvants, achieving their effects by mechanisms of **antigen** presentation and sustained **antigen** release.sup.38, have been used successfully to increase the potency of several inactivated vaccines including those against tetanus, anthrax, and serum hepatitis.sup.39,40. Though useful, alum preparations are deficient in several aspects. Control over quantity and rate of **antigen** release is limited, often resulting in a continued requirement for immunization schedules consisting of multiple injections given over a period. . .
DRWD . . . patterns and deployment of new biological warfare agents by enemy forces require flexibility in the number and types of vaccine **antigen** administered to soldiers departing for combat. Any immunization schedule requiring completion during engagement in non-linear combat would compromise this flexibility. . .
DRWD . . . hepatitis B vaccine release rate characteristics desirable for single-step immunization, (2) incorporate those release rate characteristics into a one-step biodegradable poly(DL-**lactide** -co-**glycolide**)(DL-**PLG**) microencapsulated hepatitis B surface **antigen** (HBsAg) vaccine, and (3) conduct an in vivo trial comparing the effectiveness of this single-step vaccine against the conventional three-step. . .
DRWD . . . vaccine potency assay for comparing the six-month immunization schedule currently in use.sup.41 with that of a single-step immunization by sustained **antigen** release was established according to the following protocol: Specimens for baseline antibody titers were collected from twenty mice by exsanguination.. . .
DRWD In Vitro **Antigen** Release Rate from Heptavax B vaccine
DRWD **Antigen** release from aluminum hydroxide adjuvant in HBV was measured by pumping 2 cc per hour of 1:20,000 thimerosal in saline. . . the HBsAg standards were verified by Biuret protein determination and by UV absorbance at 215 nm and 225 nm.sup.44. Nonspecific **antigen** retention on the Acrodisc filter was assessed by

measuring percent recovery of a known quantity of HBsAg. Spontaneous degradation of vaccine **antigen** was monitored by comparing daily rations of **antigen** to total protein detected in the effluent.

DRWD These studies were designed to characterize the stability of the aqueous **antigen** to the various physical conditions employed in the microencapsulation process. Conditions tested included lyophilization with reconstitution in distilled water, cyclohexane, methylene chloride, chloroform, methyl alcohol, acetone, iso-octane, hexane, acetone, pentane, or **heptane**; irradiation while lyophilized; and, exposure to elevated temperatures. Samples exposed to organic solvents were first lyophilized, reconstituted with the test. . .

DRWD Assessment of the Effect of **Antigen** Release Rate on Vaccine Potency

DRWD Microencapsulation in DL-**PLG**

DRWD Microencapsulated immunogens were fabricated by Southern Research Institute, Birmingham, Ala. DL-**PLG** polymers were synthesized from the cyclic diesters, DL **lactide** and **glycolide**, by using a ring-opening melt polymerization catalyzed by tetraphenyl tin.sup.45. The resulting polymer was dissolved i methylene chloride, filtered free of insoluble contaminants and precipitated in methanol. **Lactide**-co-**glycolide** mole ration of the product was determined by nuclear magnetic resonance spectroscopy. **Encapsulation** of HBsAg in DL:**PLG** polymer was achieved by an organic phase separation process.sup.46. Microcapsules of the desired size (approximately 100 micron diameter in these. . .

DRWD In Vitro Analysis of **Encapsulated** Antigens

DRWD Integrity of **encapsulated antigen** was assessed by comparing the **antigen** to total protein ratios present in microcapsule hydrolysates with those obtained from suspensions of pure unencapsulated **antigen**. Centrifuge tubes containing 1 ug of either microencapsulated or pure vaccine **antigen** in 1 ml saline were incubated at 4.degree. c with shaking. Samples were collected at weekly intervals by interrupting the. . . HBsAg by the Abbott Ausria II radioimmunoassay. The HBsAg standard described earlier in this report was used as the calibrator. **Antigen** destruction due to the **encapsulation** procedure was monitored by a comparison between the **antigen** assayed from the hydrolysate and from the untreated **antigen** control.

DRWD Assessment of the potency of DL:**PLG** microencapsulated HBsAg for immunizing ICR mice when used alone and in combination with Heptavax B vaccine. HBsAg loaded microcapsules that. . .

DRWD In vitro **antigen** release rate from HBV. HBsAg release from the 20 ug of Heptavax was not detected in any of the 21. . . limit of detection for the Abbott Auria II assay employed was approximately 4.8 ng/ml. The Acrodisc filter used in the **antigen** release study was back-washed with 10 mls normal saline. Quantitation of the HBsAg present within this back-wash eluent revealed the. . . obtain if there had been no deterioration of the original 40 ug/ml HBsAg loaded onto the filter, none of the **antigen** eluted from the alum adjuvant, and none of the vaccine had adsorbed onto or passed through the filter.

DRWD Evaluation of **antigen** stability. Considerable effort was expended in assessing the effects of physical conditions on the antigenicity of HBsAg to insure that. . . solution, had to be lyophilized. Initial attempts at lyophilizing HBsAg in normal saline resulted in a total loss of detectable **antigen** within samples. Dilution of the HBsAg sample 1:10 in distilled water prior to freezing resulted in reservation of nearly 100% of the **antigen** detectable in the original sample. Studies of **antigen** stability at elevated temperature revealed that HBsAg may be heated to 50.degree. C. for up to one hour without appreciable loss of **antigen**. The studies involving exposure of lyophilized **antigen** to organic solvents indicated that iso-cane and hexane

had minimal effects on antigenicity, but that 95% to 100% of antigenicity was lost upon exposure to either methylene chloride, chloroform, cyclohexane, or methyl alcohol. Moderate **antigen** loss occurred in the presence of acetone, pentane and **heptane**. As a result of these studies, hexane was chosen as the solvent for microencapsulation.

DRWD Assessment of the effect of **antigen** release rate on vaccine potency. The results (Table 5) indicated that immunogen formation (i.e., the alum adjuvant of Heptavax B). . .

DRWD HBsAg release from DL:**PLG** microcapsules. The microcapsules employed in this study were designed to disintegrate within three weeks after hydration. It is evident from. . .

DRWD Assessment of the potency of DL:**PLG** microencapsulated HBsAg for immunizing ICR mice when used alone and in combination with Heptavax B vaccine. The results (Table 6). . .

DRWD . . . be programmed during fabrication into forms that have quite difference release profiles, including slow and steady release, multiple bursts of **antigen** over a period of time, or combinations of release forms. Sieving allows choice of microcapsule size, and the ability of DL-**PLG** to sequester **antigen** from the host's immune system until release occurs enhances control over exposure of the recipient's immune system to **antigen** over a sustained period of time. These characteristics provided the impetus for these studies as they indicate potential for achieving. . .

DRWD . . . under standing of the fundamental differences between the manner in which alum and microcapsules interact with the immune system. The **antigen** release studies showed that alum firmly bound the **antigen** on its surface, whereas the microcapsules sequestered the **antigen** load within the interstices of an immunologically inert polymer. Release of **antigen** from microcapsules was spontaneous and gradual while **antigen** release from alum wa probably enzymatically mediated within host macrophages. Alum thus performed at least two useful functions as an adjuvant: by beating its entire load of **antigen** upon its surface, it provided a large single exposure of **antigen** to the host; and, by being readily phagocytized by host macrophages, it served as a means of targeting the **antigen** to the immune system.

DRWD . . . of incorporating the two properties common to alum adjuvant must be devised. These properties, which where discussed above, are targeting **antigen** to the immune system and delivering the **antigen** load in a single concentrated pulse at its target. A gradual, sustained release of free **antigen**, as was achieved with the 100 micron microcapsules used in these studies, could be expected to elicit an immune response. . .

DRWD . . . large (>10 microns in diameter) and thus fail to be readily phagocytized. In order for the larger microcapsules with prolonged **antigen** release characteristics to be efficacious, the **antigen** eventually released from those microcapsules would have be in a form which targeted and concentrated it within the recipient's immune system. This might be effectively achieved by microencapsulation of **antigen** coated alum or by microencapsulating clusters of smaller (<10 microns) microcapsules.

DRWD . . . surface to volume ratio. These smaller microcapsules would be well suited for eliciting a primary response if their pulse of **antigen** release could be programmed to occur after phagocytosis.

DRWD . . . Evans, D. G., D. J. Jr. Evans, S. Clegg, and J. A. Pauley. 1979. Purification and characterization of the CFA/I **antigen** of enterotoxigenic Escherichia coli. Infect. Immun. 25:738-748.

DRWD 10. Berzofsky, J. A. 1988. Structural basis of **antigen** recognition by T lymphocytes. J. Clin. Invest. 82:1811-1817.

DRWD . . . J. M. Bidart. 1990. Structural probing of human lutropin using antibodies raised against synthetic peptides constructed by classical and multiple **antigen** pepetide system approaches. Mol. Immunol. 27:363-368.

DRWD 17. Isaacson, R. E. 1977. K99 surface **antigen** of Escerichia coli: Purification and partial characterization. Infect. Immun. 15:272-279.

DRWD . . . D. J. Maneval, J. H. Collins, J. L. Theibert and M. M. Levine. (1989). Purification and analysis of colonization factor **antigen** I, coli surface **antigen** 1, and coli surface **antigen** 3 fimbriae from enterotoxigenic Escherichia coli. J. Bacteriol. 171, 6372-4.

DRWD . . . K., D. G. Evans, M. So and C. H. Lee. (1989). Molecurlar cloning and nucleotide sequence of the colonization factor **antigen** I gene of Escherichia coli. Infect Immun. 57, 1126-30.

DRWD . . . L.-Y., and Boedeker, E. C. Towards and oral E. Coli pilus vaccine for traveler's diarrhea: suspentibility of purified colonization factor **antigen**/II to proteolytic digestion. Gastroenterology 1985, 88, A1575.

DRWD 29. Eldridge, J. H. Gilley, R. M. Staas, J. K. Moldoveanu, Z., Meulbroek, J. A. and Tice, T. r. Biodegradable **microspheres**: vaccine delivery system for oral immunization. Curr. Top. Microbiol, Immunol. 1989, 146, 59-66.

DRWD . . . K., Gilley, R. M., and Tice, T. R. Controlled vaccine release in the gut-associated lymphoid tissue. I. Orally administered biodegradable **microsphere** target the Peyer's patches. J. Controlled release 2989, 11, 205.

DRWD . . . Eldridge, J. H. Staas, J. K., Meubroek J. A., McGhee, J. R., Tice, T. R. and Gilley, R. M. Biodegradable **microsphere** as a vaccine delivery system. Mol. Immunol, 1991, 28, 287-294.

DRWD . . . C. E., Boedeker, E. C., Reid, R. H., Jarboe, D., Wolf, M., Le, M., and Brown, W. R. Pili in **microsphere** protect rabbits for diarrhea induced by E. coli strain RDEC-1. Vaccine (in press).

DRWD . . . Jarboe, D., Reid, R., McQueen, C., and Boedeker, E., In vitro lymphocyte proliferation after sensitization or rabbit lymphoid tissue with **encapsulated** or non-**encapsulated** AF/R1 pilus adhesin of E coli strain RDED-1. Abstracts of the Annual Meeting of the American Society of Microbiology, May. . .

CLM What is claimed is:

1. An immunostimulating composition comprising **encapsulation-microspheres**, which may contain a pharmaceutically-acceptable adjuvant, wherein said **microspheres** having a diameter between 1 nanometers (nm) to 10 microns (um) are comprised of (a) a biodegradable-biocompatible poly (DL-**lactide**-co-**glycolide**) or polyglycolide as the bulk matrix, wherein the relative ratio between the amount of **lactide**; **glycolide** components are within the range of 52:48 to 0:100 and (b) an immunogenic substance comprising Colonization Factor **Antigen** and hepatitis B surface **antigen** that serves to elicit the production of antibodies and T-lymphocyte proliferation in animals.

3. An immunostimulating composition according to claim 2 wherein the relative ratio between the **lactide** and **glycolide** component is within the range of 48:52 to 58:42.

4. An immunostimulating composition according to claim 2 wherein the size of more than 50% of said **microspheres** is between 5 to 10 um in diameter by volume.

6. A vaccine comprising an immunostimulating composition of claim 5 wherein said immunogenic substance is Colony Factor **Antigen** (CFA/II).

7. A vaccine comprising an immunostimulating composition of claim 5 wherein said immunogenic substance is hepatitis B surface **antigen** (HBsAg).

11. An immunostimulating composition comprising **encapsulating-microspheres**, which may contain a pharmaceutically-acceptable adjuvant, wherein said **microspheres** having a diameter between 1 nanometers (nm) to 10 microns (um) are comprised of (a) a **glycolide** polymer as a bulk matrix and (b) an immunogenic substance comprising Colonization Factor **Antigen** and hepatitis B surface **antigen** that serves to elicit the production of antibodies and T-lymphocyte proliferation in animals.

```
=> s l6 and saccharide?
L9            1 L6 AND SACCHARIDE?

=> d l9

L9   ANSWER 1 OF 1  USPATFULL
AN     1998:64760  USPATFULL
TI     Vaccines against intracellular pathogens using antigens
       encapsulated within biodegradble-biocompatible
       microspheres
IN     Burnett, Paul R., Silver Spring, MD, United States
       Van Hamont, John E., Ft. Meade, MD, United States
       Reid, Robert H., Kensington, MD, United States
       Setterstrom, Jean A., Alpharetta, GA, United States
       Van Cott, Thomas C., Brookeville, MD, United States
       Birx, Debrah L., Potomac, MD, United States
PA     The United States of America as represented by the Secretary of the
       Army, Washington, DC, United States (U.S. government)
PI     US 5762965              19980609
AI     US 1996-598874          19960209 (8)
RLI    Continuation-in-part of Ser. No. US 1994-242960, filed on 16 May 1994
       And Ser. No. US 1995-446149, filed on 22 May 1995 which is a
       continuation of Ser. No. US 1984-590308, filed on 16 Mar 1984, now
       abandoned , said Ser. No. US   -242960 which is a continuation-in-part
       of Ser. No. US 1992-867301, filed on 10 Apr 1992, now patented, Pat. No.
       US 5417986 which is a continuation-in-part of Ser. No. US 1991-805721,
       filed on 21 Nov 1991, now abandoned which is a continuation-in-part of
       Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a
       continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990,
       now abandoned
DT     Utility
FS     Granted
LN.CNT 315
INCL   INCLM: 424/499.000
       INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/422.000
NCL    NCLM:  424/499.000
       NCLS:  424/422.000; 424/426.000; 424/455.000; 424/486.000; 424/488.000
IC     [6]
       ICM: A61K009-00
       ICS: A61K009-66; A61K009-14; A61F013-00
EXF    424/499; 424/426; 424/455; 424/486; 424/488; 424/422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> s l6 and poly saccharide?
L10           0 L6 AND POLY SACCHARIDE?

=> s l6 and carbohydrate
L11          11 L6 AND CARBOHYDRATE

=> d l11 1-11

L11  ANSWER 1 OF 11  USPATFULL
AN     2001:157849  USPATFULL
TI     Emulsion-based processes for making microparticles
IN     Gibson, John W., Springville, AL, United States
       Holl, Richard J., Indian Springs, AL, United States
       Tipton, Arthur J., Birmingham, AL, United States
PA     Southern BioSystems, Inc., Birmingham, AL, United States (U.S.
       corporation)
PI     US 6291013          B1   20010918
AI     US 1999-303842           19990503 (9)
DT     Utility
```

FS GRANTED
LN.CNT 1244
INCL INCLM: 427/213.300
INCLS: 427/231.310; 427/213.320; 427/213.330; 427/213.360; 428/402.200; 428/402.210; 264/004.100; 264/004.300; 264/004.330; 264/004.600
NCL NCLM: 427/213.300
NCLS: 427/231.310; 427/213.320; 427/213.330; 427/213.360; 428/402.200; 428/402.210; 264/004.100; 264/004.300; 264/004.330; 264/004.600
IC [7]
ICM: A61K009-16
ICS: B01J013-12
EXF 427/213.3; 427/213.31; 427/213.32; 427/213.33; 427/213.36; 428/402.2; 428/402.21; 264/4.1; 264/4.3; 264/4.33; 264/4.6

L11 ANSWER 2 OF 11 USPATFULL
AN 2001:142135 USPATFULL
TI Zace 1: a human metalloenzyme
IN Sheppard, Paul O., Granite Falls, WA, United States
PA ZymoGenetics, Inc., Seattle, WA, United States (U.S. corporation)
PI US 6280994 B1 20010828
AI US 1999-440325 19991115 (9)
DT Utility
FS GRANTED
LN.CNT 3706
INCL INCLM: 435/226.000
INCLS: 435/069.100; 435/069.700; 435/252.300; 435/252.330; 435/320.100; 536/023.200; 536/023.400
NCL NCLM: 435/226.000
NCLS: 435/069.100; 435/069.700; 435/252.300; 435/252.330; 435/320.100; 536/023.200; 536/023.400
IC [7]
ICM: C12N015-57
ICS: C12N009-64; C12N015-74; C12N015-82; C12N015-85
EXF 435/69.1; 435/69.7; 435/226; 435/252.3; 435/252.33; 435/320.1; 536/23.2; 536/23.4
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 3 OF 11 USPATFULL
AN 2000:18071 USPATFULL
TI Composition for delivering bioactive agents for immune response and its preparation
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The Uab Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 6024983 20000215
AI US 1993-116802 19930907 (8)
RLI Continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2328
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/499.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/889.000; 514/958.000; 514/963.000

NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/499.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/889.000; 514/958.000; 514/963.000
IC [7]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/237.1; 424/256.1; 424/434; 424/439; 424/497; 424/499; 424/810; 424/501; 514/885; 514/889; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 4 OF 11 USPATFULL
AN 1999:163251 USPATFULL
TI Polymeric lamellar substrate particles for drug delivery
IN Coombes, Allan Gerald Arthur, Nottingham, United Kingdom
Davis, Stanley Stewart, Nottingham, United Kingdom
Major, Diane Lisa, London, United Kingdom
Wood, John Michael, Hertsfordshire, United Kingdom
PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)
PI US 6001395 19991214
WO 9702810 19970130
AI US 1998-983156 19980330 (8)
WO 1996-GB1695 19960715
19980330 PCT 371 date
19980330 PCT 102(e) date
PRAI GB 1995-14285 19950713
DT Utility
FS Granted
LN.CNT 793
INCL INCLM: 424/501.000
INCLS: 424/426.000; 424/490.000
NCL NCLM: 424/501.000
NCLS: 424/426.000; 424/490.000
IC [6]
ICM: A61K009-16
ICS: A61K047-34
EXF 424/486; 424/426; 424/458; 424/428; 424/459; 424/490; 424/501; 514/952; 428/402; 428/402.24; 427/2.14
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 5 OF 11 USPATFULL
AN 1999:99400 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5942252 19990824
AI US 1995-469463 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2060

INCL INCLM: 424/501.000
INCLS: 424/426.000; 424/430.000; 424/434.000; 424/435.000; 424/436.000; 424/451.000; 424/464.000
NCL NCLM: 424/501.000
NCLS: 424/426.000; 424/430.000; 424/434.000; 424/435.000; 424/436.000; 424/451.000; 424/464.000
IC [6]
ICM: A61K009-50
ICS: A61K009-48; A61F002-02; A61F009-02
EXF 424/489; 424/451; 424/464; 424/490; 424/426; 424/430; 424/434; 424/435; 424/436; 424/501; 514/772.3; 514/912
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 6 OF 11 USPATFULL
AN 1999:18774 USPATFULL
TI Polymer microparticles for drug delivery
IN Yeh, Ming-Kung, Taipei, Taiwan, Province of China
Coombes, Alan Gerald, Nottingham, United Kingdom
Jenkins, Paul George, Macclesfield, United Kingdom
Davis, Stanley Stewart, Nottingham, United Kingdom
PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)
PI US 5869103 19990209
WO 9535097 19951228
AI US 1997-750738 19970404 (8)
WO 1995-GB1426 19950619
19970404 PCT 371 date
19970404 PCT 102(e) date
PRAI GB 1994-12273 19940618
DT Utility
FS Granted
LN.CNT 1058
INCL INCLM: 424/501.000
INCLS: 424/502.000; 264/004.100; 264/004.600
NCL NCLM: 424/501.000
NCLS: 264/004.100; 264/004.600; 424/502.000
IC [6]
ICM: A61K009-50
ICS: B01J013-02
EXF 424/501; 424/502; 264/4.1; 264/4.6
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 7 OF 11 USPATFULL
AN 1998:162037 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissue and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5853763 19981229
AI US 1995-467314 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted

LN.CNT 2263
INCL INCLM: 424/489.000
INCLS: 424/184.100; 424/204.100; 424/206.100; 424/234.100; 424/237.100; 424/434.000; 424/435.000; 424/436.000; 424/499.000; 424/501.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/489.000
NCLS: 424/184.100; 424/204.100; 424/206.100; 424/234.100; 424/237.100; 424/434.000; 424/435.000; 424/436.000; 424/499.000; 424/501.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/435; 424/501; 424/489; 514/888; 514/963; 514/885; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 8 OF 11 USPATFULL
AN 1998:124217 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5820883 19981013
AI US 1995-468064 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2355
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/810; 424/501; 514/888; 514/497; 514/885; 514/958; 514/963; 514/810; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 9 OF 11 USPATFULL
AN 1998:118870 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S.

corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5814344 19980929
AI US 4692187 19950606 (8)
RLI Continuation of Ser. No. 116484, filed on 7 Sep 1993 which is a continuation of Ser. No. 629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. 325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. 169973, filed on 18 Mar 1988, now patented, Pat. No. 5075109 which is a continuation-in-part of Ser. No. 923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2121
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/501; 514/499; 514/888; 514/963; 514/885; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 10 OF 11 USPATFULL
AN 1998:115447 USPATFULL
TI Method for oral or rectal delivery of microencapsulated vaccines and compositions therefor
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5811128 19980922
AI US 1164848 19930907 (8)
RLI Continuation of Ser. No. 629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. 325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. 169973, filed on 18 Mar 1988, now patented, Pat. No. 5075109 which is a continuation-in-part of Ser. No. 923159, filed on 24 Oct 1996, now abandoned
DT Utility
FS Granted
LN.CNT 2353
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 428/402.210; 428/202.240; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/501; 514/888; 514/963; 514/885; 514/958; 530/403

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 11 OF 11 USPATFULL
AN 1998:64760 USPATFULL
TI Vaccines against intracellular pathogens using antigens **encapsulated** within biodegradble-biocompatible **microspheres**
IN Burnett, Paul R., Silver Spring, MD, United States
Van Hamont, John E., Ft. Meade, MD, United States
Reid, Robert H., Kensington, MD, United States
Setterstrom, Jean A., Alpharetta, GA, United States
Van Cott, Thomas C., Brookeville, MD, United States
Birx, Debrah L., Potomac, MD, United States
PA The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
PI US 5762965 19980609
AI US 1996-598874 19960209 (8)
RLI Continuation-in-part of Ser. No. US 1994-242960, filed on 16 May 1994 And Ser. No. US 1995-446149, filed on 22 May 1995 which is a continuation of Ser. No. US 1984-590308, filed on 16 Mar 1984, now abandoned , said Ser. No. US -242960 which is a continuation-in-part of Ser. No. US 1992-867301, filed on 10 Apr 1992, now patented, Pat. No. US 5417986 which is a continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990, now abandoned
DT Utility
FS Granted
LN.CNT 315
INCL INCLM: 424/499.000
INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/422.000
NCL NCLM: 424/499.000
NCLS: 424/422.000; 424/426.000; 424/455.000; 424/486.000; 424/488.000
IC [6]
ICM: A61K009-00
ICS: A61K009-66; A61K009-14; A61F013-00
EXF 424/499; 424/426; 424/455; 424/486; 424/488; 424/422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic l11 11

L11 ANSWER 11 OF 11 USPATFULL
TI Vaccines against intracellular pathogens using antigens **encapsulated** within biodegradble-biocompatible **microspheres**
AB This invention relates to parenteral and mucosal vaccines against diseasesaused by intracellular pathogens using antigens **encapsulated** within a biodegradable-biocompatible **microspheres**(matrix).
SUMM This invention relates to parenteral and mucosal vaccines against diseases caused by intracellular pathogens using antigens **encapsulated** within biodegradable-biocompatible **microspheres**(matrix).
SUMM The issues of durability and mucosal immunogenicity have been previously addressed by **encapsulating** vaccine antigens in appropriately-sized biodegradable, biocompatible **microspheres** made of **lactide/glycolide** copolymer (the same materials used in resorbable sutures). It has been shown that such **microspheres** can be made to release their load in a controlled manner over a prolonged period of time and can facilitate. . .
SUMM . . . on the surface of both free virus and infected cells, and present it to the immune system (systemic and mucosal)

encapsulated in **microspheres** to protect and augment its immunogenicity.

DETD This invention relates to a novel pharmaceutical composition, a microcapsule/sphere formulation, which comprises an **antigen** **encapsulated** within a biodegradable polymeric matrix, such as poly(DL-**lactide** co **glycolide**) (**PLG**), wherein the relative ratio between the **lactide** and **glycolide** component of the **PLG** is within the range of 52:48 to 0:100, and its use, as a vaccine, in the effective induction of antiviral. . . . antigens. In the practice of this invention, applicants found that when a complex (oligomeric) native envelope protein of HIV-1 was **encapsulated** in **PLG** **microspheres**, it retained its native antigenicity and function upon its release in vitro. Furthermore, when used as a vaccine in animals,. . . .

DETD Microencapsulation of immunogens: **PLG microspheres** ranging from 1 to 20 um in diameter and containing a 0.5 to 1.0% **antigen** core load were prepared by a solvent extractive method. The solvent extraction method involves dissolving the viral **antigen** and sucrose (1:4 ratio w:w) in 1 ml of deionized water. This solution is flash frozen and lyophilized. The resulting **antigen**-loaded sucrose particles are resuspended in acetonitrile and mixed into **PLG** copolymer dissolved in acetonitrile. This **antigen**-polymer mixture is then emulsifyed into heavy mineral oil, transferred into heptane and mixed for 30 min to extract the oil. . . . from the nascent spheres. The spheres are harvested by centrifugation, washed three times in heptane and dried overnight under vacuum. **Microsphere** size was determined by both light and scanning electron microscopy. The **antigen** core load was determined by quantitative amino acid analysis of the **microspheres** following complete hydrolysis in 6N hydrochloric acid.

DETD Analysis of immunogen spontaneously released from **microspheres** in vitro by binding to soluble CD4 and recognition by HIV-positive patient serum. **PLG microspheres** loaded with native (oligomeric) gp 160 were suspended in phosphate-buffered saline, pH 7.4 (PBS), incubated at 37 C. for 3 h, and then at 4 C overnight. The **microspheres** were then sedimented by centrifugation (2 min at 200.times.g), the supernatants harvested, and the released gp 160 assayed for binding. . . .

DETD Immunization of animals. HIV-seronegative, 8-10 week old NZW rabbits were immunized intramuscularly with rgp 160- or o-gp 160-loaded **PLG microspheres** suspended in PBS or with alum-adjuvanted rgp 160 in PBS. Groups receiving rgp 160-loaded **microspheres** (n=2) were primed with 50 ug of immunogen on day 0 and boosted with 25 ug on day 42. Groups receiving o-gp 160-loaded **microspheres** (n=3) were primed with 70 ug of immunogen on day 0 and boosted with 35 ug on day 56. Groups. . .

DETD BALB/c mice were immunized subcutaneously with rgp 160-loaded **PLG microspheres** suspended in PBS or with alum-adjuvanted rgp 160 in PBS. The mice in all groups (n=4) received 10 ug of. . .

DETD Comparison of the native (oligomeric) gp 160 prior to microencapsulation and following spontaneous release from **PLG** **microspheres** showed the two to be essentially indistinguishable in terms of their binding to CD4 and recognition by HIV-positive patient serum. (Table 1). This retention of conformation-dependent binding shows that structure of the **antigen** is not appreciably altered by the microencapsulation process.

DETD . . . (CTL) assay performed on the speen cells of mice which had had been previously immunized with either HIV-1 envelope protein **encapsulated** in **PLG microspheres** (dark squares) or the same protein administered in a conventional way with alum adjuvant (dark diamonds). These data indicate that

microencapsulation of HIV-1 envelope protein in **PLG microspheres** results in a vaccine that induces significantly greater anti-HIV CTL activity than does alum-adjuvanted vaccine. The open symbol groups represent. . .

DETD . . . binding of antibodies to native vs denatured viral protein. These data show that rabbits immunized with a non-native HIV-1 protein **encapsulated** in **PLG** (#5 and 6) develop antibodies which show greater binding to denatured (vs native) protein (indicated by a ratio<1). On the other hand, rabbits immunized with a native HIV-1 protein **encapsulated** in **PLG microspheres** (#10-12) develop antibodies which show greater binding to native viral protein (indicated by ratio>1). This retention of each proteins antigenicity constitutes an additional piece of evidence that the structure of antigens loaded in **PLG microspheres** are preserved.

DETD Microencapsulation of immunogens: **PLG microspheres** ranging from 1 to 15 um in diameter and containing a 0.5 to 1.0% **antigen** core load were prepared by a solvent evaporation method. The solvent evaporation method involves emulsifying the viral **antigen** dissolved in deionized water into poly(DL-**lactide**-co-**glycolide**) polymer dissolved in methylene chloride. This emulsion is mixed into 0.9% polyvinyl alcohol and stirred. After 10 min of stirring,. . . 1.5 h. The resulting spheres are harvested by centrifugation, washed three times in distilled water, and dried overnight under vacuum. **Microsphere** size was determined by both light and scanning electron microscopy. The **antigen** core load was determined by quantitative amino acid analysis of the **microspheres** following complete hydrolysis in 6N hydrochloric acid.

DETD Analysis of spontaneously released **antigen** showed it to retain its CD4 binding capacity. Its native antigenicity (recognition by the serum of an HIV-positive patient) was only slightly less than that of the **antigen** prior to **encapsulation** and following spontaneous release from **microspheres** produced by a solvent extraction method (Table 1).

DETD The results of immunizing animals with either non-native (denatured) or native oligomeric gp 160 in **PLG microspheres** produced by a solvent evaporation method were essentially indistinguishable from those obtained using **microspheres** produced by a solvent extraction method (example 1). Microencapsulated **antigen** induced significantly greater CTL activity than **antigen** administered in a conventional alum-adjuvanted formulation. Furthermore, preservation of the structure of **PLG** -microencapsulated antigens is supported by the findings of preferential binding of antibodies elicited by **microspheres** loaded with denatured **antigen** to denatured gp 120 (FIGS. 2, 3 and 4) and the preferred binding of antibodies elicited by **microspheres** loaded with native (oligomeric) **antigen** to native gp 120 (FIGS. 2, 7-8).

DETD TABLE 1

BIA (released o-gp160)
Capture o-gp160-451 (stock vs **microsphere**-released)
on tvc 391 fc3/fc4 sCD4 (4 mg/m)
1 ul/min flow rate for o-gp160 inj.; 5 ul/min for all others

Ilgate	RU	HIV+/sCD4 (RU ratio)
gp120-MN 1:10	3286	
HIV+ 1:100	54	
NHS 1:100	3	
HIV+ pool 1:100	47	
o-gp160 (tvc281)	1772	

HIV+	3259	1.84
tvc281	1848	
NHS	-36	
tvc281	1762	
HIV+ pool	2597	1.47
tvc281-**PLG**-EV	3342	
HIV+	4594	1.37
tvc281	3222	
NHS	7	
tvc281	3210	
HIV+ pool	3336	1.04
tvc281-**PLG**-EX	1855	
HIV+	3760	2.04
tvc281	1839	
NHS	2	
tvc281	1850	
HIV+ pool	2745	1.48
gp120-MN 1:10	2914	
HIV+ 1:100	14	
NHS 1:100	-2	
HIV+ pool 1:100	14	
tvc281	1099	
HIV+	1083	0.99
tvc281	1022	
HIV+ pool	1395	1.36
tvc281-**PLG**-EV	1595	
HIV+	1322	0.83
tvc281	1535	
HIV+ pool	1781	1.16

CLM What is claimed is:

1. An immunostimulating composition comprising **encapsulating microspheres** comprised of (a) a biodegradable-biocompatible poly(DL-**lactide**-co-**glycolideas** the bulk matrix produced by a solvent evaporation process wherein the molecular weight of the copolymer is between 4,000 to. . .

2. The immunostimulating composition described in claim 1 wherein the **antigen** is pre-**encapsulated** into a conformationally stabilizing hydrophilic matrix consisting of an appropriate mono, di- or tri-saccharide or other **carbohydrate** susbstance by lyophilization prior to its final **encapsulation** into the **PLG microsphere** by a solvent extraction process employing acetonitrile as the polymer solvent, mineral oil as the emulsion's external phase, and heptane. . .

3. The immunostimulating compositions described in claims 1 or 2 wherein the immunogenic substance is a native (oligomeric)HIV-1 envelope **antigen** that is conformationally stabilized by the polymer matrix and serves to elicit in animals the production of HIV specific cytotoxic T lumphocytes and antibodies preferentially reactive against native HIV-1 envelope **antigen**.

5. The immunostimulating compositions describe in claim 4 wherein the relative ratio between the amount of the **lactide**: **glycolide** components of said matrix is within the range of 52:48 to 0:100.

. . . immunostimulating compositions described in claim 5, employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . in claim 5, employed as a mucosal vaccine wherein the size of more than 50% (by volume) of said vaccine **microspheres** is between 5 to 10 microns in diameter.

10. A composition in accordance with claim 1 wherein the **microspheres** further contain a pharmaceutically-acceptable adjuvant.

. . . immunostimulating compositions described in claim 6 employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . immunostimulating compositions described in claim 7 employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . in claim 6 employed as a mucosal vaccine wherein the size of more than 50% (by volume) of said vaccine **microspheres** is between 5 to 10 microns in diameter.

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=> s l6 and saccharide?
L9            1 L6 AND SACCHARIDE?

=> d l9

L9   ANSWER 1 OF 1  USPATFULL
AN     1998:64760  USPATFULL
TI     Vaccines against intracellular pathogens using antigens
       encapsulated within biodegradble-biocompatible
       microspheres
IN     Burnett, Paul R., Silver Spring, MD, United States
       Van Hamont, John E., Ft. Meade, MD, United States
       Reid, Robert H., Kensington, MD, United States
       Setterstrom, Jean A., Alpharetta, GA, United States
       Van Cott, Thomas C., Brookeville, MD, United States
       Birx, Debrah L., Potomac, MD, United States
PA     The United States of America as represented by the Secretary of the
       Army, Washington, DC, United States (U.S. government)
PI     US 5762965              19980609
AI     US 1996-598874          19960209 (8)
RLI    Continuation-in-part of Ser. No. US 1994-242960, filed on 16 May 1994
       And Ser. No. US 1995-446149, filed on 22 May 1995 which is a
       continuation of Ser. No. US 1984-590308, filed on 16 Mar 1984, now
       abandoned , said Ser. No. US   -242960 which is a continuation-in-part
       of Ser. No. US 1992-867301, filed on 10 Apr 1992, now patented, Pat. No.
       US 5417986 which is a continuation-in-part of Ser. No. US 1991-805721,
       filed on 21 Nov 1991, now abandoned which is a continuation-in-part of
       Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a
       continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990,
       now abandoned
DT     Utility
FS     Granted
LN.CNT 315
INCL   INCLM: 424/499.000
       INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/422.000
NCL    NCLM:  424/499.000
       NCLS:  424/422.000; 424/426.000; 424/455.000; 424/486.000; 424/488.000
IC     [6]
       ICM: A61K009-00
       ICS: A61K009-66; A61K009-14; A61F013-00
EXF    424/499; 424/426; 424/455; 424/486; 424/488; 424/422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> s l6 and poly saccharide?
L10           0 L6 AND POLY SACCHARIDE?

=> s l6 and carbohydrate
L11          11 L6 AND CARBOHYDRATE

=> d l11 1-11

L11  ANSWER 1 OF 11  USPATFULL
AN     2001:157849  USPATFULL
TI     Emulsion-based processes for making microparticles
IN     Gibson, John W., Springville, AL, United States
       Holl, Richard J., Indian Springs, AL, United States
       Tipton, Arthur J., Birmingham, AL, United States
PA     Southern BioSystems, Inc., Birmingham, AL, United States (U.S.
       corporation)
PI     US 6291013         B1   20010918
AI     US 1999-303842          19990503 (9)
DT     Utility
```

FS GRANTED
LN.CNT 1244
INCL INCLM: 427/213.300
INCLS: 427/231.310; 427/213.320; 427/213.330; 427/213.360; 428/402.200; 428/402.210; 264/004.100; 264/004.300; 264/004.330; 264/004.600
NCL NCLM: 427/213.300
NCLS: 427/231.310; 427/213.320; 427/213.330; 427/213.360; 428/402.200; 428/402.210; 264/004.100; 264/004.300; 264/004.330; 264/004.600
IC [7]
ICM: A61K009-16
ICS: B01J013-12
EXF 427/213.3; 427/213.31; 427/213.32; 427/213.33; 427/213.36; 428/402.2; 428/402.21; 264/4.1; 264/4.3; 264/4.33; 264/4.6

L11 ANSWER 2 OF 11 USPATFULL
AN 2001:142135 USPATFULL
TI Zace 1: a human metalloenzyme
IN Sheppard, Paul O., Granite Falls, WA, United States
PA ZymoGenetics, Inc., Seattle, WA, United States (U.S. corporation)
PI US 6280994 B1 20010828
AI US 1999-440325 19991115 (9)
DT Utility
FS GRANTED
LN.CNT 3706
INCL INCLM: 435/226.000
INCLS: 435/069.100; 435/069.700; 435/252.300; 435/252.330; 435/320.100; 536/023.200; 536/023.400
NCL NCLM: 435/226.000
NCLS: 435/069.100; 435/069.700; 435/252.300; 435/252.330; 435/320.100; 536/023.200; 536/023.400
IC [7]
ICM: C12N015-57
ICS: C12N009-64; C12N015-74; C12N015-82; C12N015-85
EXF 435/69.1; 435/69.7; 435/226; 435/252.3; 435/252.33; 435/320.1; 536/23.2; 536/23.4
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 3 OF 11 USPATFULL
AN 2000:18071 USPATFULL
TI Composition for delivering bioactive agents for immune response and its preparation
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The Uab Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 6024983 20000215
AI US 1993-116802 19930907 (8)
RLI Continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2328
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/499.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/889.000; 514/958.000; 514/963.000

NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/499.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/889.000; 514/958.000; 514/963.000
IC [7]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/237.1; 424/256.1; 424/434; 424/439; 424/497; 424/499; 424/810; 424/501; 514/885; 514/889; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 4 OF 11 USPATFULL
AN 1999:163251 USPATFULL
TI Polymeric lamellar substrate particles for drug delivery
IN Coombes, Allan Gerald Arthur, Nottingham, United Kingdom
Davis, Stanley Stewart, Nottingham, United Kingdom
Major, Diane Lisa, London, United Kingdom
Wood, John Michael, Hertsfordshire, United Kingdom
PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)
PI US 6001395 19991214
WO 9702810 19970130
AI US 1998-983156 19980330 (8)
WO 1996-GB1695 19960715
19980330 PCT 371 date
19980330 PCT 102(e) date
PRAI GB 1995-14285 19950713
DT Utility
FS Granted
LN.CNT 793
INCL INCLM: 424/501.000
INCLS: 424/426.000; 424/490.000
NCL NCLM: 424/501.000
NCLS: 424/426.000; 424/490.000
IC [6]
ICM: A61K009-16
ICS: A61K047-34
EXF 424/486; 424/426; 424/458; 424/428; 424/459; 424/490; 424/501; 514/952; 428/402; 428/402.24; 427/2.14
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 5 OF 11 USPATFULL
AN 1999:99400 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5942252 19990824
AI US 1995-469463 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2060

INCL INCLM: 424/501.000
INCLS: 424/426.000; 424/430.000; 424/434.000; 424/435.000; 424/436.000; 424/451.000; 424/464.000
NCL NCLM: 424/501.000
NCLS: 424/426.000; 424/430.000; 424/434.000; 424/435.000; 424/436.000; 424/451.000; 424/464.000
IC [6]
ICM: A61K009-50
ICS: A61K009-48; A61F002-02; A61F009-02
EXF 424/489; 424/451; 424/464; 424/490; 424/426; 424/430; 424/434; 424/435; 424/436; 424/501; 514/772.3; 514/912
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 6 OF 11 USPATFULL
AN 1999:18774 USPATFULL
TI Polymer microparticles for drug delivery
IN Yeh, Ming-Kung, Taipei, Taiwan, Province of China
Coombes, Alan Gerald, Nottingham, United Kingdom
Jenkins, Paul George, Macclesfield, United Kingdom
Davis, Stanley Stewart, Nottingham, United Kingdom
PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)
PI US 5869103 19990209
WO 9535097 19951228
AI US 1997-750738 19970404 (8)
WO 1995-GB1426 19950619
19970404 PCT 371 date
19970404 PCT 102(e) date
PRAI GB 1994-12273 19940618
DT Utility
FS Granted
LN.CNT 1058
INCL INCLM: 424/501.000
INCLS: 424/502.000; 264/004.100; 264/004.600
NCL NCLM: 424/501.000
NCLS: 264/004.100; 264/004.600; 424/502.000
IC [6]
ICM: A61K009-50
ICS: B01J013-02
EXF 424/501; 424/502; 264/4.1; 264/4.6
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 7 OF 11 USPATFULL
AN 1998:162037 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissue and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5853763 19981229
AI US 1995-467314 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted

LN.CNT 2263
INCL INCLM: 424/489.000
INCLS: 424/184.100; 424/204.100; 424/206.100; 424/234.100; 424/237.100; 424/434.000; 424/435.000; 424/436.000; 424/499.000; 424/501.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/489.000
NCLS: 424/184.100; 424/204.100; 424/206.100; 424/234.100; 424/237.100; 424/434.000; 424/435.000; 424/436.000; 424/499.000; 424/501.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/435; 424/501; 424/489; 514/888; 514/963; 514/885; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 8 OF 11 USPATFULL
AN 1998:124217 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally-associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5820883 19981013
AI US 1995-468064 19950606 (8)
RLI Continuation of Ser. No. US 1993-116484, filed on 7 Sep 1993 which is a continuation of Ser. No. US 1990-629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. US 1988-169973, filed on 18 Mar 1988, now patented, Pat. No. US 5075109 which is a continuation-in-part of Ser. No. US 1986-923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2355
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/810; 424/501; 514/888; 514/497; 514/885; 514/958; 514/963; 514/810; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 9 OF 11 USPATFULL
AN 1998:118870 USPATFULL
TI Method for delivering bioactive agents into and through the mucosally associated lymphoid tissues and controlling their release
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S.

corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5814344 19980929
AI US 4692187 19950606 (8)
RLI Continuation of Ser. No. 116484, filed on 7 Sep 1993 which is a continuation of Ser. No. 629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. 325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. 169973, filed on 18 Mar 1988, now patented, Pat. No. 5075109 which is a continuation-in-part of Ser. No. 923159, filed on 24 Oct 1986, now abandoned
DT Utility
FS Granted
LN.CNT 2121
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/501; 514/499; 514/888; 514/963; 514/885; 514/958; 530/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 10 OF 11 USPATFULL
AN 1998:115447 USPATFULL
TI Method for oral or rectal delivery of microencapsulated vaccines and compositions therefor
IN Tice, Thomas R., Birmingham, AL, United States
Gilley, Richard M., Birmingham, AL, United States
Eldridge, John H., Birmingham, AL, United States
Staas, Jay K., Birmingham, AL, United States
PA Southern Research Institute, Birmingham, AL, United States (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
PI US 5811128 19980922
AI US 1164848 19930907 (8)
RLI Continuation of Ser. No. 629138, filed on 18 Dec 1990, now abandoned which is a continuation-in-part of Ser. No. 325193, filed on 16 Mar 1989, now abandoned which is a continuation-in-part of Ser. No. 169973, filed on 18 Mar 1988, now patented, Pat. No. 5075109 which is a continuation-in-part of Ser. No. 923159, filed on 24 Oct 1996, now abandoned
DT Utility
FS Granted
LN.CNT 2353
INCL INCLM: 424/501.000
INCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 428/402.210; 428/202.240; 514/885.000; 514/888.000; 514/963.000
NCL NCLM: 424/501.000
NCLS: 424/237.100; 424/256.100; 424/497.000; 424/810.000; 428/402.210; 428/402.240; 514/885.000; 514/888.000; 514/963.000
IC [6]
ICM: A61K009-52
ICS: A61K039-085; A61K039-12; A61K039-39
EXF 428/402.21; 428/402.24; 424/439; 424/461; 424/499; 424/237.1; 424/256.1; 424/434; 424/497; 424/810; 424/501; 514/888; 514/963; 514/885; 514/958; 530/403

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 11 OF 11 USPATFULL
AN 1998:64760 USPATFULL
TI Vaccines against intracellular pathogens using antigens **encapsulated** within biodegradble-biocompatible **microspheres**
IN Burnett, Paul R., Silver Spring, MD, United States
Van Hamont, John E., Ft. Meade, MD, United States
Reid, Robert H., Kensington, MD, United States
Setterstrom, Jean A., Alpharetta, GA, United States
Van Cott, Thomas C., Brookeville, MD, United States
Birx, Debrah L., Potomac, MD, United States
PA The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
PI US 5762965 19980609
AI US 1996-598874 19960209 (8)
RLI Continuation-in-part of Ser. No. US 1994-242960, filed on 16 May 1994 And Ser. No. US 1995-446149, filed on 22 May 1995 which is a continuation of Ser. No. US 1984-590308, filed on 16 Mar 1984, now abandoned , said Ser. No. US -242960 which is a continuation-in-part of Ser. No. US 1992-867301, filed on 10 Apr 1992, now patented, Pat. No. US 5417986 which is a continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-690485, filed on 24 Apr 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990, now abandoned
DT Utility
FS Granted
LN.CNT 315
INCL INCLM: 424/499.000
INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/422.000
NCL NCLM: 424/499.000
NCLS: 424/422.000; 424/426.000; 424/455.000; 424/486.000; 424/488.000
IC [6]
ICM: A61K009-00
ICS: A61K009-66; A61K009-14; A61F013-00
EXF 424/499; 424/426; 424/455; 424/486; 424/488; 424/422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic l11 11

L11 ANSWER 11 OF 11 USPATFULL
TI Vaccines against intracellular pathogens using antigens **encapsulated** within biodegradble-biocompatible **microspheres**
AB This invention relates to parenteral and mucosal vaccines against diseasesaused by intracellular pathogens using antigens **encapsulated** within a biodegradable-biocompatible **microspheres** (matrix).
SUMM This invention relates to parenteral and mucosal vaccines against diseases caused by intracellular pathogens using antigens **encapsulated** within biodegradable-biocompatible **microspheres** (matrix).
SUMM The issues of durability and mucosal immunogenicity have been previously addressed by **encapsulating** vaccine antigens in appropriately-sized biodegradable, biocompatible **microspheres** made of **lactide/glycolide** copolymer (the same materials used in resorbable sutures). It has been shown that such **microspheres** can be made to release their load in a controlled manner over a prolonged period of time and can facilitate. . .
SUMM . . . on the surface of both free virus and infected cells, and present it to the immune system (systemic and mucosal)

encapsulated in **microspheres** to protect and augment its immunogenicity.

DETD This invention relates to a novel pharmaceutical composition, a microcapsule/sphere formulation, which comprises an **antigen** **encapsulated** within a biodegradable polymeric matrix, such as poly(DL-**lactide** co **glycolide**) (**PLG**), wherein the relative ratio between the **lactide** and **glycolide** component of the **PLG** is within the range of 52:48 to 0:100, and its use, as a vaccine, in the effective induction of antiviral. . . . antigens. In the practice of this invention, applicants found that when a complex (oligomeric) native envelope protein of HIV-1 was **encapsulated** in **PLG** **microspheres**, it retained its native antigenicity and function upon its release in vitro. Furthermore, when used as a vaccine in animals,. . .

DETD Microencapsulation of immunogens: **PLG microspheres** ranging from 1 to 20 um in diameter and containing a 0.5 to 1.0% **antigen** core load were prepared by a solvent extractive method. The solvent extraction method involves dissolving the viral **antigen** and sucrose (1:4 ratio w:w) in 1 ml of deionized water. This solution is flash frozen and lyophilized. The resulting **antigen**-loaded sucrose particles are resuspended in acetonitrile and mixed into **PLG** copolymer dissolved in acetonitrile. This **antigen**-polymer mixture is then emulsifyed into heavy mineral oil, transferred into heptane and mixed for 30 min to extract the oil. . . from the nascent spheres. The spheres are harvested by centrifugation, washed three times in heptane and dried overnight under vacuum. **Microsphere** size was determined by both light and scanning electron microscopy. The **antigen** core load was determined by quantitative amino acid analysis of the **microspheres** following complete hydrolysis in 6N hydrochloric acid.

DETD Analysis of immunogen spontaneously released from **microspheres** in vitro by binding to soluble CD4 and recognition by HIV-positive patient serum. **PLG microspheres** loaded with native (oligomeric) gp 160 were suspended in phosphate-buffered saline, pH 7.4 (PBS), incubated at 37 C. for 3 h, and then at 4 C overnight. The **microspheres** were then sedimented by centrifugation (2 min at 200.times.g), the supernatants harvested, and the released gp 160 assayed for binding. . .

DETD Immunization of animals. HIV-seronegative, 8-10 week old NZW rabbits were immunized intramuscularly with rgp 160- or o-gp 160-loaded **PLG microspheres** suspended in PBS or with alum-adjuvanted rgp 160 in PBS. Groups receiving rgp 160-loaded **microspheres** (n=2) were primed with 50 ug of immunogen on day 0 and boosted with 25 ug on day 42. Groups receiving o-gp 160-loaded **microspheres** (n=3) were primed with 70 ug of immunogen on day 0 and boosted with 35 ug on day 56. Groups. . .

DETD BALB/c mice were immunized subcutaneously with rgp 160-loaded **PLG microspheres** suspended in PBS or with alum-adjuvanted rgp 160 in PBS. The mice in all groups (n=4) received 10 ug of. . .

DETD Comparison of the native (oligomeric) gp 160 prior to microencapsulation and following spontaneous release from **PLG** **microspheres** showed the two to be essentially indistinguishable in terms of their binding to CD4 and recognition by HIV-positive patient serum. (Table 1). This retention of conformation-dependent binding shows that structure of the **antigen** is not appreciably altered by the microencapsulation process.

DETD . . . (CTL) assay performed on the speen cells of mice which had had been previously immunized with either HIV-1 envelope protein **encapsulated** in **PLG microspheres** (dark squares) or the same protein administered in a conventional way with alum adjuvant (dark diamonds). These data indicate that

microencapsulation of HIV-1 envelope protein in **PLG microspheres** results in a vaccine that induces significantly greater anti-HIV CTL activity than does alum-adjuvanted vaccine. The open symbol groups represent. . .

DETD . . . binding of antibodies to native vs denatured viral protein. These data show that rabbits immunized with a non-native HIV-1 protein **encapsulated** in **PLG** (#5 and 6) develop antibodies which show greater binding to denatured (vs native) protein (indicated by a ratio<1). On the other hand, rabbits immunized with a native HIV-1 protein **encapsulated** in **PLG microspheres** (#10-12) develop antibodies which show greater binding to native viral protein (indicated by ratio>1). This retention of each proteins antigenicity constitutes an additional piece of evidence that the structure of antigens loaded in **PLG microspheres** are preserved.

DETD Microencapsulation of immunogens: **PLG microspheres** ranging from 1 to 15 um in diameter and containing a 0.5 to 1.0% **antigen** core load were prepared by a solvent evaporation method. The solvent evaporation method involves emulsifying the viral **antigen** dissolved in deionized water into poly(DL-**lactide**-co-**glycolide**) polymer dissolved in methylene chloride. This emulsion is mixed into 0.9% polyvinyl alcohol and stirred. After 10 min of stirring,. . . 1.5 h. The resulting spheres are harvested by centrifugation, washed three times in distilled water, and dried overnight under vacuum. **Microsphere** size was determined by both light and scanning electron microscopy. The **antigen** core load was determined by quantitative amino acid analysis of the **microspheres** following complete hydrolysis in 6N hydrochloric acid.

DETD Analysis of spontaneously released **antigen** showed it to retain its CD4 binding capacity. Its native antigenicity (recognition by the serum of an HIV-positive patient) was only slightly less than that of the **antigen** prior to **encapsulation** and following spontaneous release from **microspheres** produced by a solvent extraction method (Table 1).

DETD The results of immunizing animals with either non-native (denatured) or native oligomeric gp 160 in **PLG microspheres** produced by a solvent evaporation method were essentially indistinguishable from those obtained using **microspheres** produced by a solvent extraction method (example 1). Microencapsulated **antigen** induced significantly greater CTL activity than **antigen** administered in a conventional alum-adjuvanted formulation. Furthermore, preservation of the structure of **PLG** -microencapsulated antigens is supported by the findings of preferential binding of antibodies elicited by **microspheres** loaded with denatured **antigen** to denatured gp 120 (FIGS. 2, 3 and 4) and the preferred binding of antibodies elicited by **microspheres** loaded with native (oligomeric) **antigen** to native gp 120 (FIGS. 2, 7-8).

DETD TABLE 1

BIA (released o-gp160) Capture o-gp160-451 (stock vs **microsphere**-released) on tvc 391 fc3/fc4 sCD4 (4 mg/m) 1 ul/min flow rate for o-gp160 inj.; 5 ul/min for all others		
Ilgate	RU	HIV+/sCD4 (RU ratio)
gp120-MN 1:10	3286	
HIV+ 1:100	54	
NHS 1:100	3	
HIV+ pool 1:100	47	
o-gp160 (tvc281)	1772	

HIV+	3259	1.84
tvc281	1848	
NHS	-36	
tvc281	1762	
HIV+ pool	2597	1.47
tvc281-**PLG**-EV	3342	
HIV+	4594	1.37
tvc281	3222	
NHS	7	
tvc281	3210	
HIV+ pool	3336	1.04
tvc281-**PLG**-EX	1855	
HIV+	3760	2.04
tvc281	1839	
NHS	2	
tvc281	1850	
HIV+ pool	2745	1.48
gp120-MN 1:10	2914	
HIV+ 1:100	14	
NHS 1:100	-2	
HIV+ pool 1:100	14	
tvc281	1099	
HIV+	1083	0.99
tvc281	1022	
HIV+ pool	1395	1.36
tvc281-**PLG**-EV	1595	
HIV+	1322	0.83
tvc281	1535	
HIV+ pool	1781	1.16

CLM What is claimed is:

1. An immunostimulating composition comprising **encapsulating microspheres** comprised of (a) a biodegradable-biocompatible poly(DL-**lactide**-co-**glycolideas** the bulk matrix produced by a solvent evaporation process wherein the molecular weight of the copolymer is between 4,000 to. . .

2. The immunostimulating composition described in claim 1 wherein the **antigen** is pre-**encapsulated** into a conformationally stabilizing hydrophilic matrix consisting of an appropriate mono, di- or tri-saccharide or other **carbohydrate** susbstance by lyophilization prior to its final **encapsulation** into the **PLG microsphere** by a solvent extraction process employing acetonitrile as the polymer solvent, mineral oil as the emulsion's external phase, and heptane. . .

3. The immunostimulating compositions described in claims 1 or 2 wherein the immunogenic substance is a native (oligomeric)HIV-1 envelope **antigen** that is conformationally stabilized by the polymer matrix and serves to elicit in animals the production of HIV specific cytotoxic T lumphocytes and antibodies preferentially reactive against native HIV-1 envelope **antigen**.

5. The immunostimulating compositions describe in claim 4 wherein the relative ratio between the amount of the **lactide**: **glycolide** components of said matrix is within the range of 52:48 to 0:100.

. . . immunostimulating compositions described in claim 5, employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . in claim 5, employed as a mucosal vaccine wherein the size of more than 50% (by volume) of said vaccine **microspheres** is between 5 to 10 microns in diameter.

10. A composition in accordance with claim 1 wherein the **microspheres** further contain a pharmaceutically-acceptable adjuvant.

. . . immunostimulating compositions described in claim 6 employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . immunostimulating compositions described in claim 7 employed as a parentally administered vaccine wherein the diameter size range of said vaccine **microspheres** lies between 1 nanometer and 20 microns.

. . . in claim 6 employed as a mucosal vaccine wherein the size of more than 50% (by volume) of said vaccine **microspheres** is between 5 to 10 microns in diameter.